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STRUCTURE *of* ANTIGENS

M.H.V. *Van Regenmortel*



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PREFACE

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The structural basis of antigenic determinants is of considerable interest to biologists for two reasons. One reason is that it is expected that an elucidation of the structure will provide considerable light on the general principles of antigenicity and specificity. Thus, the first step in understanding how the molecules amidst the enormous complexity of biological systems arise from the realization that there are many applications in medicine. Such applications are the preparation of vaccines, the structure, conformation, and development of new vaccines, and the study of infectious agents.

In the present two-volume work, the analytical methods used to elucidate the antigenic determinants of molecular entities, recognizable combining sites, several chapters are devoted to the structure of antibodies. Antibodies are relational partners that can bind to each other. The occurrence of a binding reaction is a functional concept and not specifically a recognition phenomena, the latter being associated with measurements of binding affinity. Measurement is always subject to error, so it is important to consider which physicochemical methods are most appropriate for immunoassay. Therefore, several chapters are devoted to the development of modern immunoassays.

Twelve of the chapters are concerned with physicochemical principles. Some chapters are concerned with some specific applications (e.g., functional activity and biological activity, allergens, etc.) or by their association with specific antigens of microorganisms, viruses, and other agents.

The book is intended for students and teachers of biological science who wish to understand the principles of antigenic specificity.

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Chapter

1

Molecular Dissection of Protein Antigens

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ANTIGENIC DETERMINANTS OF PROTEINS

The antigenic specificity of a protein resides in restricted areas of the molecule, known as antigenic determinants or epitopes, which are recognized by the combining sites or paratopes of certain immunoglobulin molecules. The precision of steric and chemical fit between epitope and paratope necessary for achieving this type of immunological "recognition" is highly variable since different antibodies may have affinity constants in the range of 10^3 to 10^{11} L/mol.

The most common way of classifying epitopes consists in distinguishing continuous and discontinuous epitopes (Atassi and Smith, 1978). The label continuous epitope is given to any short linear peptide fragment of the antigen that is able to bind to antibodies raised against the intact protein. Usually these antibodies cross-react only weakly with the peptide and the continuous epitope identified in this manner is unlikely to mimic exactly the conformation and structure of the corresponding epitope in the intact protein. The peptide fragment probably does not retain the conformation present in the folded protein, and, furthermore, it is likely to represent only a portion of a more complex epitope.

The second type of epitope, known as discontinuous epitope, is believed to correspond to the vast majority of epitopes found in proteins. They are made up of residues that are not continuous in sequence but are brought

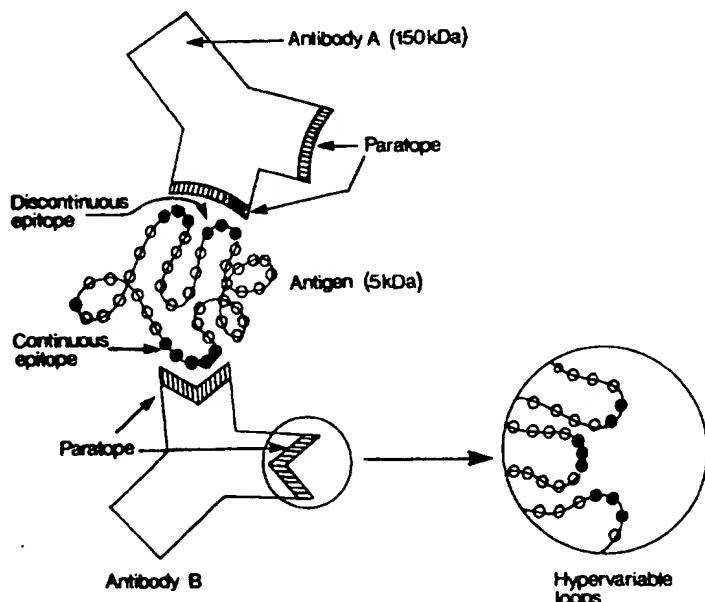


Figure 1. Schematic representation of two antibodies interacting with a continuous and a discontinuous epitope of a protein antigen. Contact residues are indicated in black. (From Van Regenmortel, 1986.)

together by the folding of the polypeptide chain (Fig. 1). Most antibodies to discontinuous epitopes will bind to the protein only if the molecule is intact and its conformation is preserved. When the protein is fragmented into a number of peptides, the various residues that made up the discontinuous epitope are scattered and each component is no longer individually recognized by the antibody. It is generally accepted today that the majority of monoclonal antibodies (MAbs) raised against intact proteins are specific for discontinuous epitopes, and it is believed that this explains why such MAbs usually do not react with any linear peptide fragment derived from the antigen. However, it is also true that a certain percentage of the MAbs raised against intact proteins, usually of the order of 10%, do react with linear peptide fragments of the protein. Since the range of specificities observed with a panel of MAbs is very similar to that found in a polyclonal antiserum to the same antigen (Quesniaux et al., 1990), it is reasonable to assume that about 10% of the antibodies present in an antiserum are also able to recognize so-called continuous epitopes of the protein antigen.

In recent years, some authors have challenged the view that *native* proteins possess continuous epitopes. Laver et al. (1990), for instance, suggested that all continuous epitopes represent "unfoldons," i.e., unfolded regions of the antigen that cross-react only with antibodies specific for the denatured protein. Such antibodies may be present in antisera raised against the protein because

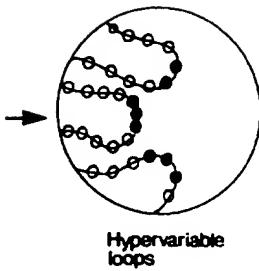
some of the antigen molecules are unfolded (Bielski, 1973; Lando and Rabinowich, 1973; Lando and Rabinowich, 1974). These antibodies are obtained by immunizing animals with unfolded proteins and may recognize the presence of denatured protein in a radioimmunoassay. Although it is true that the protein antigen is at least partially unfolded, it is an extreme view to maintain that all proteins and peptides are denatured forms of the protein. The most likely explanation for the ability of some antibodies to bind to peptides of many different viruses, it is believed, is that binding with peptides can lead to the formation of infectious particles (Anderer and Schlegel, 1986; Emini et al., 1986; Roehrig et al., 1989; Tamm, 1988). The antipeptide antibodies recognize infectious particles. Such antibodies may also bind to the cognate structure in the protein antigen, reproducing exactly the epitope specificity of the antibodies.

Although only a small fraction of antibodies to native proteins leads to the formation of infectious particles, there is considerable interest in the development of applications that would arise in the use of antipeptide antibodies (Lerner, 1984; Walt et al., 1988).

ANTIGENIC CROSS-REACTIVITY

Antigenic cross-reactivity between an antigen and its homologs or variants may be observed because two or more identical epitopes may be present in a polyclonal antiserum. This type of cross-reactivity may be described as *shared reactivity*, since the antibody recognizes the same epitope in different antigens. In the present context (Berzofsky et al., 1986), a panel of MAbs raised against two antigenically related proteins may react in an identical fashion, i.e., they may recognize the same epitope (cross-reactivity), which has been described as *specific cross-reactivity*.

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es interacting with a continuous contact residues are indicated in

n (Fig. 1). Most antibodies to only if the molecule is intact protein is fragmented into a t made up the discontinuous longer individually recognized that the majority of monoclonal are specific for discontinuous by such MAbs usually do not I from the antigen. However, e MAbs raised against intact with linear peptide fragments observed with a panel of MAbs antiserum to the same antigen assume that about 10% of the le to recognize so-called con-

ed the view that *native* proteins (i), for instance, suggested that i.e., unfolded regions of the cific for the denatured protein. ed against the protein because

some of the antigen molecules used for immunization were denatured (Sci-bienski, 1973; Lando and Reichlin, 1982; Jemmerson, 1987a). Similarly, antibodies obtained by immunization with linear peptides may also be specific for unfoldons and may recognize the parent protein only because of the presence of denatured protein molecules in the preparation used in the immunoassay. Although it is true that in many immunoassays in current use, the protein antigen is at least partly denatured, it seems nevertheless too extreme a view to maintain that all reported cases of cross-reactivity between proteins and peptides are due to antibodies specific only for the denatured form of the protein. The most compelling argument against this view lies in the ability of some anti-peptide antibodies to neutralize certain biological activities associated with the native state of proteins. For instance, in the case of many different viruses, it has been firmly established that immunization with peptides can lead to the formation of antibodies that neutralize virus infectivity (Anderer and Schlumberger, 1965; Bittle et al., 1982; DiMarchi et al., 1986; Emini et al., 1985; McCray and Werner, 1987; Parry et al., 1988; Roehrig et al., 1989; Smyth et al., 1990). These findings imply that the anti-peptide antibodies recognize the native state of the viral protein present in infectious particles. Such findings do not mean that the linear peptide reproduces exactly the epitope in the intact protein but only that it resembles the cognate structure in the parent protein sufficiently to allow antibody cross-reactivity.

Although only a small fraction of the total immune response against a protein leads to the formation of antibodies that cross-react with linear peptides, there is considerable interest in unravelling the structural basis of this cross-reactivity. The main impetus for these studies lies in the many practical applications that would arise if protein antigens could be replaced by synthetic peptides (Lerner, 1984; Walter, 1986).

ANTIGENIC CROSS-REACTIVITY

Antigenic cross-reactivity is a consequence of the fact that the relationship between an antigen and its antibody is never of an exclusive nature. Cross-reactivity may be observed because two multideterminant antigens share one or more identical epitopes recognized by distinct antibody subpopulations present in a polyclonal antiserum. This type of cross-reactivity, which may be described as *shared reactivity*, arises because a particular antibody recognizes the same epitope in two different proteins and is of little relevance in the present context (Berzofsky and Schechter, 1981). For instance, when a panel of MAbs raised against a multideterminant protein is tested against two antigenically related proteins, it is frequently observed that certain MAbs react in an identical fashion with the two antigens, obviously because they recognize the same epitope (Briand et al., 1982). A totally different type of cross-reactivity, which has been termed *true cross-reactivity* (Berzofsky and

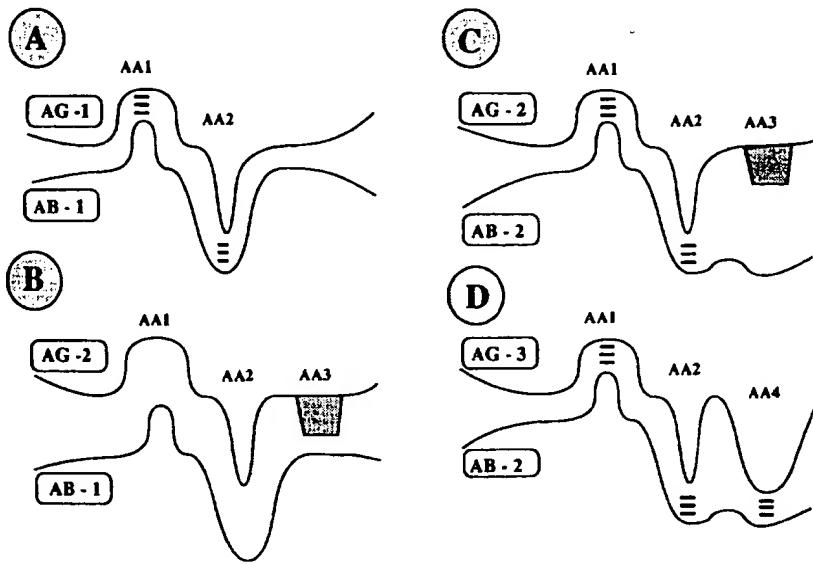


Figure 2. Schematic representation of epitope-paratope interfaces. In A, the energetic epitope of antigen 1 consists of two residues (AA1 and AA2) hydrogen-bonded to residues of the paratope of antibody 1. In B, the same energetic epitope of antigen 1 seems to be present, but a bulky substitution in a third residue (AA3) prevents H-bond formation with antibody 1. The influence of a substitution of AA3 might lead to the conclusion that this residue is implicated in the epitope of antigen 1, although its contribution is limited to the establishment of shape complementarity. In C, the energetic epitope in antigen 2 is again found to interact once the necessary complementarity in shape has been reestablished with antibody 2. In D, antibody 2 is able to show increased (i.e., heterospecific) binding with the modified epitope in antigen 3.

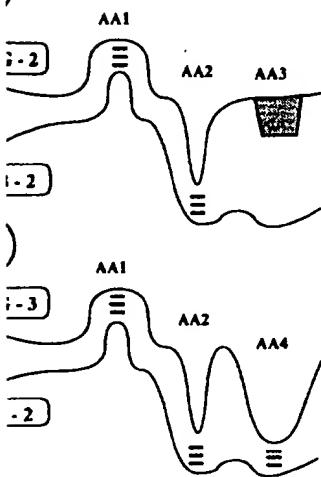
Schechter, 1981), arises when a particular antibody recognizes an epitope that is different but structurally related to the epitope used to raise the antibody. In most cases, the paratope will react with higher affinity with the homologous epitope used for immunization, although it is not uncommon for a paratope to bind more strongly to certain other epitopes than to the one against which it was raised (see Fig. 2). This phenomenon, known as heterospecificity (or heteroclitic binding), is commonly found whenever it is looked for, i.e., when the antibody is tested against a range of antigens closely related structurally to the immunogen (Al Moudallal et al., 1982; Harper et al., 1987; Mäkelä, 1965; Underwood, 1985).

True cross-reactivity occurs, for instance, when a peptide fragment of a protein cross-reacts with an epitope of the intact protein. Since the initial protein conformation present before fragmentation is unlikely to be retained in a peptide fragment, an antibody raised against the protein reacts with the peptide with a lower affinity. In addition, the linear peptide fragment usually represents only part of a more complex discontinuous epitope, which means that there will be fewer contact points between the paratope and the cross-

reacting peptide than with the mentality determining region: some contact residues to the I possible that a smaller numbe with the cross-reactive peptid

When there are only few the possibility arises that the a particular epitope will be ab case the antibody, by the invo will be able to cross-react wit whatsoever. Such cross-reacti MAbs sometimes react in a reactivity is enhanced when of repeated epitopes or when immunoblotting assays (Ghos local density of epitopes fav which facilitates the detection of low affinity. When IgM : further amplified (Hirayama reactions that are not based or common (Ghosh and Campb

The study f cross-reacti fragments has led to the identif epitopes of proteins. Not eve that is believed to constitute a interacting with the paratope. can be replaced by any of t antigenic reactivity of the pep peptide is, in fact, antigenicall continuous and discontinuo mortel, 1987). There is no of residues necessary for gi described as immunological r Obviously, the minimum num evidence that a highly accessi with a certain degree of specil 1966). This should come as n capable of rec gnizing as sm possible adjacent pairings of Geysen et al. (1986) have sh recognize some of these ami detect such antibody reactivity bivalent antibody binding is a low intrinsic affinity for th is extended at both ends by :



interfaces. In A, the energetic epitope gen-bonded to residues of the paratope in 1 seems to be present, but a bulky nation with antibody 1. The influence his residue is implicated in the epitope establishment of shape complementarity. 2 interact once the necessary comple-

2. In D, antibody 2 is able to show epitope in antigen 3.

body recognizes an epitope that is used to raise the antibody. higher affinity with the homologous protein is not uncommon for a paratope than to the one against which known as heterospecificity (or ever it is looked for, i.e., when closely related structurally; Harper et al., 1987; Mäkelä,

when a peptide fragment of a intact protein. Since the initial ation is unlikely to be retained in the protein reacts with the linear peptide fragment usually in situ us epitope, which means on the paratope and the cross-

reacting peptide than with the homologous protein. Whereas all six complementarity determining regions (CDR) of the antibody may have contributed some contact residues to the paratope that recognized the intact protein, it is possible that a smaller number of CDRs are involved in the binding observed with the cross-reactive peptide.

When there are only few contact points between paratope and epitope, the possibility arises that the CDRs that are not involved in the binding with a particular epitope will be able to bind to a totally dissimilar epitope. In this case the antibody, by the involvement of different subregions of the paratope, will be able to cross-react with two epitopes that have no structural similarity whatsoever. Such cross-reactions are probably responsible for the finding that MAbs sometimes react in a nonspecific manner with certain antigens. This reactivity is enhanced when the antigen presents a high local concentration of repeated epitopes or when it is used at a high density in solid phase or immunoblotting assays (Ghosh and Campbell, 1986). In both cases, the high local density of epitopes favors bivalent binding with IgG-type antibodies which facilitates the detection of weak cross-reactions shown by antibodies of low affinity. When IgM antibodies are used, the multivalence effect is further amplified (Hirayama et al., 1985), which makes irrelevant cross-reactions that are not based on a structural similarity in the epitope even more common (Ghosh and Campbell, 1986).

The study of cross-reactions between intact proteins and linear peptide fragments has led to the identification of large numbers of so-called continuous epitopes of proteins. Not every residue in the linear stretch of 5 to 8 residues that is believed to constitute a continuous epitope is, in fact, a contact residue interacting with the paratope. Usually some residues in the continuous epitope can be replaced by any of the other 19 amino acids without impairing the antigenic reactivity of the peptide (Geysen, 1985). This means that the linear peptide is, in fact, antigenically discontinuous and that the distinction between continuous and discontinuous epitopes is somewhat artificial (Van Regenmortel, 1987). There is no consensus at present about the minimum number of residues necessary for giving rise to a process that could properly be described as immunological recognition as opposed to chemical recognition. Obviously, the minimum number is one residue and there is, in fact, published evidence that a highly accessible, single C-terminal residue can be recognized with a certain degree of specificity by antibodies (Anderer and Schlumberger, 1966). This should come as no surprise in view of the existence of antibodies capable of recognizing as small a structure as dinitrophenol. There are 400 possible adjacent pairings of the 20 common amino acids, and studies by Geysen et al. (1986) have shown that certain MAbs are able to preferentially recognize some of these amino acid pairs. Since the immunoassay used to detect such antibody reactivity employs a high density of rod-coupled peptides, bivalent antibody binding is favored. This allows antibodies that have only a low intrinsic affinity for the dipeptide to be detected. When the dipeptide is extended at both ends by additional residues, some of the tetra- and hex-

apeptides that are obtained will be recognized by the MAb even better. When a particular residue is added to the dipeptide, it is sometimes found that it can be placed at more than one position and still increase the binding reaction. For instance, Geysen et al. (1986) reported that a MAb, which reacted weakly with the dipeptide Met-Lys, showed increasingly better reactivity with Try-Met-Lys, with Try-Met-Lys-His, and even better with Try-Gln-Met-Arg-His-Ser. This type of result implies that a variety of contacts can be established between epitope and paratope residues and that peptides of increasing size are able to readjust themselves to different subregions of the paratope (Edmundson et al., 1987).

In view of the multispecificity of antibodies, there is no guarantee that building up an epitope in this way (a "mimotope" according to Geysen's terminology) will produce a structure that is antigenically equivalent to the immunogen used to raise the test antibody. Until now there is no good evidence that immunization with such mimotopes produces antibodies able to recognize the structure being mimicked.

There is evidence that sequences of three residues can be recognized by certain antibodies of low affinity (Geysen et al., 1986; Trifilieff et al., 1991). It is also possible that longer peptides of 4 to 8 residues bind to an antibody because of the presence in the peptide of a few contact residues interspersed with noninteracting residues playing the role of scaffold. In such a case the systematic replacement of each residue of the peptide by other amino acids would show that the three contact residues are essential for binding while the other residues may be replaceable by virtually any amino acid (Schoofs et al., 1988). The minimum number of contacts or chemical bonds necessary for specific antibody recognition cannot be defined in an *a priori* manner. Furthermore, the minimum level of affinity required for calling the binding of an immunoglobulin "specific" and for considering that such an immunoglobulin is an "antibody" for the ligand depends on the context and on the type of assay used (Van Regenmortel, 1989a).

At the other end of the scale, it is equally meaningless to speak of perfect fit between epitope and paratope. It is clearly impossible to rule out that an antibody may show improved heterospecific binding with a structural relative of the epitope. In practice, it seems that *discrimination potential* is a more useful concept than *specificity* for describing the binding pattern of an antibody. It is the particular need of the investigator to differentiate between two entities that provides the necessary criterion for deciding whether an antibody is specific or not. Specificity is only meaningful with respect to a desired level of discrimination and this depends on the particular task at hand. The same antibody may thus be called specific or nonspecific, depending on the context.

Methods Used	
Method	Type Re-
X-ray crystallography of antigen-Fab complexes	Dis- re- ge-
Use of peptide fragments as cross-reactive antigenic probes	Co- cr- ht
1. Free peptides 2. Peptides adsorbed to solid-phase 3. Peptides conjugated to carrier 4. Peptides attached to support used for synthesis	
Identification of critical residues in peptide fragment by systematic replacement studies	Co- co- re- w- di
Use of fusion proteins and peptides	Co
1. Prokaryotic expression vectors 2. Chimeras	
Use of anti-peptide antibodies	Co- ci- hi
Study of mutants, analogs and homologous proteins	Di
Topographic mapping by competitive binding assay	Or- o

METHODS USED FO

Methods used for identification of epitopes have been described in several reviews (Atassi, 1984; Trifilieff et al., 1991; Edmundson and Paterson, 1986; Geysen et al., 1986). The approaches that have been used are summarized in Table I. Elsewhere (Van Regenmortel, 1989a), a structural analysis is presented. This analysis describes the spatial arrangement of the surfaces and tends to emphasize the fact that a stable complex has been formed.

TABLE 1.
Methods Used for Localizing Protein Epitopes

Method	Type of Epitope Recognized	Criterion for Residue Allocation
X-ray crystallography of antigen-Fab complexes	Discontinuous epitope reacting with homologous antibody	Contact with epitope-paratope interface
Use of peptide fragments as cross-reactive antigenic probes	Continuous epitope cross-reacting with heterologous antibody	Residual binding of linear fragment above threshold of assay
1. Free peptides 2. Peptides adsorbed to solid-phase 3. Peptides conjugated to carrier 4. Peptides attached to support used for synthesis		
Identification of critical residues in peptide fragment by systematic replacement studies	Continuous epitope containing essential residues interspersed with irrelevant residues	Abrogation or decrease of cross-reactivity by replacement of functionally essential residue
Use of fusion proteins and peptides	Continuous epitope	Residual binding above threshold of assay
1. Prokaryotic expression vectors 2. Chimeras		
Use of antipeptide antibodies	Continuous epitope cross-reacting with heterologous antibody	Induction of cross-reactive antibodies
Study of mutants, analogs and homologous proteins	Discontinuous epitopes	Abrogation or decrease of cross-reactivity
Topographic mapping by competitive binding assay	Only relative position of epitopes is defined	

METHODS USED FOR LOCALIZING EPITOPEs

Methods used for identifying epitopes in proteins have been described in several reviews (Atassi, 1984; Benjamin et al., 1984; Berzofsky, 1985; Jemmerson and Paterson, 1986; Van Regenmortel, 1984, 1989a). The different approaches that have been used are summarized in Table 1. As discussed elsewhere (Van Regenmortel, 1989b), the only method that is truly based on a structural analysis is X-ray crystallography of antigen-antibody complexes. This analysis describes the spatial relationships observed at the two interacting surfaces and tends to emphasize the static aspects of the contact points once a stable complex has been formed. It should be noted that the identification

of a contact residue contributing to the structure of an epitope is not at all straightforward since different definitions of what constitutes a contact have been given (Getzoff et al., 1988). For instance, contact residues can be defined as residues within Van der Waals contact, residues buried to a certain radius probe sphere, or residues with their side chains interacting directly. The five epitopes that have so far been analyzed by X-ray crystallography were found to comprise 15 to 22 contact residues, a size considerably larger than what has been traditionally regarded as being the size of an epitope, i.e., 5 to 6 residues.

All other methods listed in Table 1 are based on binding measurements and introduce the fourth dimension of time as a component of what is being observed. Binding assays take the form of activity measurements and correspond to a functional analysis of antigen-antibody interaction (Van Regenmortel, 1989b). Such analysis incorporates dynamic aspects that are not directly perceptible in a structural picture describing the bound state at equilibrium. Furthermore, most methods based on binding assays listed in Table 1 analyze the cross-reactive binding properties of antibodies, i.e., the ability of antibodies to cross-react with related structures that may differ considerably in conformation from the intact homologous antigen used to raise the antibody. Therefore, much of the information that is obtained in this way relates to rather incomplete and adulterated versions of the original epitopes existing in the native protein. These methods have led to the conclusion that only 3 to 8 residues of the antigen are critical to antibody binding. The epitope defined in a functional sense thus appears to involve fewer residues than the epitope defined in structural terms. Recently, an attempt has been made to compute how many of the residues allocated to various epitopes actually contribute to the binding energy of interaction (Novotny et al., 1989). By calculating the relative binding contributions of individual residues in the epitope defined in structural terms, it was found that most of the free energy contribution came from as few as 3 to 5 residues. This makes it possible to define a so-called "energetic epitope" in which the energetics of complex formation are emphasized and which has a size similar to that of epitopes defined by binding assays. The additional residues present in the structural epitope can then be viewed as playing a scaffolding role necessary to keep the critical, interacting residues in their proper position and orientation.

An important conclusion, therefore, is that different analytical approaches lead to different perceptions of what constitutes a protein epitope. As illustrated schematically in Fig. 2, an energetic epitope may, for instance, be viewed as comprising two residues (AA1 and AA2 in Fig. 2A) which are hydrogen-bonded to residues of the paratope. Although both residues are retained in antigen 2 (Fig. 2B), a bulky substitution at AA3 located outside the energetic epitope would eliminate the binding potential of the energetic epitope for antibody 1. If the epitope had been mapped in terms of which substitutions affect binding, residue AA3 would have been included in the epitope of antigen 1. On the other hand, when tested with respect to antibody

2, the energetic epitope in furthermore, another substitutio heterospecific binding by an lational partners that should in the absolute. Like all relat exists only by virtue of its r a particular paratope.

Crystallographic Analysis

During the last 5 years, antibody interaction has been studies of complexes of mon antigens (Colman, 1988; Da epitopes have been analyzed 1986; Padlan et al., 1989; S minidase (Colman et al., 19 area of the protein surface amino acid residues was ide antibody combining site. In shape between the interacting almost entirely excluded fro

All epitopes identified 1 continuous. The epitopes rec 5 antibodies consist essentia chain (residues 18 to 27 and for HyHEL-5), whereas the exposed surface of a helix several lysozyme segments The two neuraminidase epit consists of segments 368 t to 350 (Colman et al., 1987 ments of the polypeptide ch

The three lysozyme par of the antibody, whereas t from only four CDRs (antil addition, some framework r tion.

In the lysozyme-antibo were observed in the lysozym movement of the backbone have been recorded (Davie such small adjustments are i for antigen-antibody interacti and-key" model (Mariuzza

ire of an epitope is not at all what constitutes a contact have contact residues can be defined dues buried to a certain radius interacting directly. The five by crystallography were found considerably larger than what size of an epitope, i.e., 5 to 6

sed on binding measurements a component of what is being tivity measurements and cor-body interaction (Van Regen-namic aspects that are not di; the bound state at equilibrium. ssays listed in Table 1 analyze dies, i.e., the ability of anti-ut may differ considerably in gen used to raise the antibody. btained in this way relates to the original epitopes existing to the conclusion that only 3 ntibody binding. The epitope involve fewer residues than the an attempt has been made to l to various epitopes actually n (Novotny et al., 1989). By of individual residues in the d that most of the free energy uses. This makes it possible to ich the energetics of complex ze similar to that of epitopes idues present in the structural olding role necessary to keep position and orientation. different analytical approaches es a protein epitope. As illus-epitope may, for instance, be AA2 in Fig. 2A) which are . Although both residues are tution at AA3 located outside ng potential of the energetic en mapped in terms of which ld have been included in the tested with respect to antibody

2, the energetic epitope in antigen 2 is again able to bind (Fig. 2C). Furthermore, another substitution (AA4 in antigen 3, Fig. 2D) may give rise to heterospecific binding by antibody 2. Clearly, epitope-paratope pairs are relational partners that should be defined only in terms of each other and not in the absolute. Like all relational concepts (e.g., father, brother) an epitope exists only by virtue of its relationship with a complementary partner, i.e., a particular paratope.

Crystallographic Analysis of Protein-Antibody Complexes

During the last 5 years, our knowledge of the structural basis of antigen-antibody interaction has been considerably advanced by X-ray diffraction studies of complexes of monoclonal antibody Fab fragments with their protein antigens (Colman, 1988; Davies et al., 1988; Mariuzza et al., 1987). Five epitopes have been analyzed by this method, three of lysozyme (Amit et al., 1986; Padlan et al., 1989; Sheriff et al., 1987) and two of influenza neuraminidase (Colman et al., 1987; Tulip et al., 1989). In all five cases, a large area of the protein surface (700 to 800 Å², comprising between 15 to 22 amino acid residues was identified as being in contact with residues of the antibody combining site. In all cases there was so much complementarity in shape between the interacting surfaces of antigen and antibody that water was almost entirely excluded from the interface (see Chapter 2).

All epitopes identified by X-ray crystallography so far are clearly discontinuous. The epitopes recognized by the antilysozyme D1.3 and HyHEL-5 antibodies consist essentially of two stretches of the lysozyme polypeptide chain (residues 18 to 27 and 116 to 129 for D1.3, and 41 to 53 and 67 to 70 for HyHEL-5), whereas the lysozyme HyHEL-10 epitope consists of the exposed surface of a helix (residues 88 to 99) together with residues from several lysozyme segments (residues 15 to 16, 20 to 21, 63, and 74 to 75). The two neuraminidase epitopes are also discontinuous. The NC41 epitope consists of segments 368 to 370, 400 to 403, 430 to 434 and portions of 325 to 350 (Colman et al., 1987), whereas the NC10 epitope involves five segments of the polypeptide chain.

The three lysozyme paratopes are made up of residues from all six CDRs of the antibody, whereas the two neuraminidase paratopes utilize residues from only four CDRs (antibody NC10) or five CDRs (antibody NC41). In addition, some framework residues are also involved in the binding interaction.

In the lysozyme-antibody complexes, no large conformational changes were observed in the lysozyme as a result of the binding to antibody. However, movement of the backbone atoms of as much as 2 Å at the point of contact have been recorded (Davies et al., 1988). It is a matter of debate whether such small adjustments are in favor of the "induced fit" mechanism proposed for antigen-antibody interaction or whether they are compatible with the "lock-and-key" model (Mariuzza et al., 1987; Van Regenmortel, 1989a). It is clear

that readjustment of side chains does occur but movement of the backbone atoms is more difficult to establish unambiguously. The magnitude of the motions observed in the segmental mobility of the peptide chain is only of the order of 1 to 2 Å and the functional significance of such small movements is a matter of interpretation. Recently, a small rearrangement (0.5 to 0.7 Å) of the V_H and V_L domains of the paratope of anti-lysozyme antibody D1.3 was found to occur upon complex formation with the antigen and this was interpreted in terms of induced fit (Bhat et al., 1990).

The two explanatory models of antigen-antibody interaction, the lock-and-key and induced fit models, are sometimes presented as mutually exclusive, although it is clear that both models are useful to describe some aspects of epitope-paratope recognition. In a similar vein, the opposition between static and dynamic views of antigenicity seems somewhat artificial (Novotny et al., 1987a). The debate on whether the location of epitopes in proteins is better "explained" by the static surface accessibility of certain regions or by their segmental mobility (Novotny et al., 1986; Sasaki et al., 1988; Tainer et al., 1985; Westhof et al., 1984) is clouded by the fact that the accessibility and mobility of short segments of polypeptide chains are not independent variables but represent interconnected aspects of the folding pattern of globular proteins. Loops and turns, for instance, are mostly surface projections and also tend to possess higher than average mobility. Attempts to find the best correlation between these properties and the antigenicity of ill-defined "continuous" epitopes have given rise to much debate and contradictory claims (Geysen et al., 1987a; Hopp, 1986; Novotny et al., 1987b; Thornton et al., 1986). Unfortunately, the search for correlations has not yet produced an effective method for predicting the location of epitopes in proteins.

Preliminary X-ray data of crystal structures of complexes of peptides with anti-peptide Fab have been published (Schulze-Gahmen et al., 1988; Stura et al., 1989). In one study, the detailed structure of a complex between peptide 67 to 89 of myohemerythrin and a Fab fragment obtained by immunization with the peptide was presented (Stanfield et al., 1990). Surprisingly, the N-terminal part of the peptide was found to adopt a β -turn conformation in the antibody-peptide complex, although the same region in native myohemerythrin was in a helical conformation. However, since the Mab used to prepare the Fab had been screened for reactivity with solid-phase plate-bound myohemerythrin (Fieser et al., 1987) it is possible that the helical conformation was absent in the plate-bound antigen. The authors reported that solution-phase myohemerythrin was able to compete with the solid-phase protein in ELISA and suggested that this unusual finding was brought about by a conformational change due to "unknown causes" (Stanfield et al., 1990). It is not clear to what extent induced fit phenomena may be responsible for these observations (Crumpton, 1986; Wilson et al., 1985).

Studies with Peptides as

The most widely used in identifying which natural or able to cross-react with an peptide that is able to bind continuous epitope. Peptide which is the smallest pepti reactivity (Benjamini, 1977 served with small peptides is corresponding to chain term ments are surface-oriented (mobile than internal sector explain why the cross-reacti to be much higher than with i 1977; Milton and Van Reger Westhof et al., 1984).

Free Peptides and Peptide

As a result of rapid deve synthesis (Atherton and She Lewis, 1985; Plaué, 1990; V have virtually replaced natu synthesis, the peptides are c peptides in solution, as imm as peptide-carrier conjugate determine the antigenic rea (Van Regenmortel et al., reactivity of peptides is hig 1986). Sometimes the free Van Regenmortel, 1982; N activity is higher when the et al., 1985). In such cases t protein probably induces th for antibody recognition. It sponding to inner sequences at their termini which are a peptide bonds. The presenc any potential antigenic cros hand, when the free α -carbo an amide group, the antigeni (Gras-Masse et al., 1986; H fore, that the detection of adversely affected both if t or if the charges are remov

ut movement of the backbone iously. The magnitude of the f the peptide chain is only of ance of such small movements rearrangement (0.5 to 0.7 Å) f antilysozyme antibody D1.3 with the antigen and this was , 1990).

ntibody interaction, the lock-s presented as mutually exclu- seful to describe some aspects vein, the opposition between somewhat artificial (Novotny ition of epitopes in proteins is bility of certain regions or by 6; Sasaki et al., 1988; Tainer y the fact that the accessibility le chains are not independent f the folding pattern of globular mostly surface projections and lity. Attempts to find the best tigenicity of ill-defined "con- bate and contradictory claims et al., 1987b; Thornton et al., ions has not yet produced an epitopes in proteins.

f complexes of peptides with Gahmen et al., 1988; Stura et of a complex between peptide ent obtained by immunization ., 1990). Surprisingly, the N- & a β -turn conformation in the e region in native myoheme- , since the Mab used to prepare solid-phase plate-bound myo- that the helical conformation authors reported that solution- with the solid-phase protein in z was brought about by a con- (Stanfield et al., 1990). It is a may be responsible for these 1985).

Studies with Peptides as Antigenic Probes

The most widely used method for localizing protein epitopes consists in identifying which natural or synthetic peptide fragments of the molecule are able to cross-react with antibodies raised against the intact protein. Any peptide that is able to bind to the protein antibodies is said to contain a continuous epitope. Peptides of decreasing size may be tested to determine which is the smallest peptide that retains a significant level of antigenic reactivity (Benjamini, 1977). The degree of antigenic cross-reactivity observed with small peptides is mostly very low with the exception of peptides corresponding to chain termini. In a majority of proteins, the terminal segments are surface-oriented (Thornton and Sibanda, 1983) and are also more mobile than internal sections of the polypeptide chain. Both features may explain why the cross-reactivity observed with short terminal peptides tends to be much higher than with internal peptides (Absolom and Van Regenmortel, 1977; Milton and Van Regenmortel, 1979; Tainer et al., 1985; Walter, 1986; Westhof et al., 1984).

Free Peptides and Peptide Conjugates

As a result of rapid developments in the technique of solid-phase peptide synthesis (Atherton and Sheppard, 1989; Houghten, 1985; Kent and Clark-Lewis, 1985; Plaué, 1990; Van Regenmortel et al., 1988), synthetic peptides have virtually replaced natural peptide fragments as antigenic probes. After synthesis, the peptides are cleaved from the resin and are tested either as free peptides in solution, as immobilized peptides adsorbed to a solid phase, or as peptide-carrier conjugates. The different immunoassay formats used to determine the antigenic reactivity of peptides have recently been reviewed (Van Regenmortel et al., 1988). It should be stressed that the antigenic reactivity of peptides is highly dependent on the assay format (Muller et al., 1986). Sometimes the free peptide in solution is most active (Altschuh and Van Regenmortel, 1982; Nestorowicz et al., 1985), but in other cases the activity is higher when the peptide is conjugated to a carrier (Al Moudallal et al., 1985). In such cases the microenvironment at the surface of the carrier protein probably induces the peptide to adopt a more suitable conformation for antibody recognition. It should also be recognized that peptides corresponding to inner sequences of a polypeptide chain possess ionizable groups at their termini which are absent in the protein because of the formation of peptide bonds. The presence of the additional charged groups could lower any potential antigenic cross-reactivity with the intact protein. On the other hand, when the free α -carboxyl group of the terminal COOH is replaced by an amide group, the antigenicity of the peptide may also be altered drastically (Gras-Masse et al., 1986; Hodges et al., 1988). It must be recognized, therefore, that the detection of cross-reactivity with the parent protein can be adversely affected both if the extraneous charges of the peptide are retained or if the charges are removed by amidation or acetylation.

The binding of a peptide to antiprotein antibodies may be facilitated by the induction of a native-like conformation in the peptide during formation of the peptide-antibody complex (Crumpton, 1986; Getzoff et al., 1987, 1988). The use of longer peptides does not necessarily lead to a higher level of cross-reactivity since longer peptides may adopt a conformation different from that present in the native protein (Jemmerson, 1987b; Wilson et al., 1984). Shorter peptides may also fold more easily into the proper orientation required for binding to the antibody (Hodges et al., 1988). A variety of approaches have been used to increase the level of conformational mimicry between peptide and intact protein (see Chapter 3). Cyclization of the peptide has frequently been used for this purpose (Arnon et al., 1971; Dorow et al., 1985; Dreesman et al., 1982; Fourquet et al., 1988; Jemmerson and Hutchinson, 1990; Schulze-Gahmen et al., 1986), although it appears that information on the three-dimensional structure of the epitope is required to achieve the best results (Muller et al., 1990; Plaué, 1990). Other strategies for stabilizing certain peptide backbone conformations have also been proposed (Gras-Masse et al., 1988; Mutter, 1988; Satterthwait et al., 1989).

The antigenic cross-reactivity of peptides may be tested by measuring their capacity to inhibit the reaction of the protein with its homologous antibodies, or simply by adsorbing the peptides to a solid phase and measuring their ability to be recognized by antiprotein antibodies. In the latter case, it may be necessary to try a variety of buffers (Geerligs et al., 1988) and to prevent the peptide solution from drying up during the test (Norrby et al., 1987). It has been suggested that in order to establish the specific nature of a cross-reaction, it is necessary to include controls in which the liquid-phase protein is allowed to compete with plate-bound antigen and to show that inhibition to a level approaching 100% occurs (Jemmerson, 1987a). However, it cannot be excluded that a genuinely cross-reacting antigen reacts with antibodies so weakly that a complete inhibition cannot be observed at experimentally achievable concentrations (Berzofsky and Schechter, 1981).

Peptides Attached to Support Used for Synthesis

Peptides can be tested for antigenic activity without prior cleavage from the support used during synthesis (Hurrell et al., 1977; Shi et al., 1984). The pepscan method developed by Geysen et al. (1984, 1987b) allows the concurrent synthesis of hundreds of peptides on polyethylene pins. The pins are assembled into a polyethylene holder with the format and spacing of a microtiter plate. This allows the peptides to be tested by an enzyme immunoassay while they remain attached to the pin. After each test the pins can be freed of bound antibody by sonication and retested with different antibody preparations as many as 30 times. The pepscan method is ideally suited for the systematic testing of all possible overlapping peptides of a protein, starting from the N-terminal residue down to the C-terminal one. Usually 6 to 10 residue-long peptides are analyzed in this fashion (Geysen et al., 1987b). Covalent attachment of the peptide to the solid support may, in some cases,

impair its antigenic activity that would be revealed as an assay. Another problem is that some peptide sequences tend to facilitate the detection of vicinal dipeptide sequences may be technique (Geysen et al., 1984).

Identification of Critical Residues

The pepscan technique involves the substitution of individual amino acid residues by replacement sets in which each of the other 19 possible amino acids is essential for binding since they impair the antigenic reaction. The residues that contribute to the replaced by all common amino acid may be limited to that of a side-chain linked to retention of antibodies, Geysen et al. (1984). The residues in hexapeptides were

Studies with Fusion Proteins

Epitopes can be identified in a eukaryotic expression system as a fusion product (Lenstra et al., 1986). In this system, the product of an insertion portion of a hybrid galactosidase/hybrid protein is solubilized and applied to a nitrocellulose filter for two years a number of expression systems (1991; Mehra et al., 1986; Yoh et al., 1986). The epitopes of one virus are expressed in the particles of another virus (Delpeyroux et al., 1986; Michel et al., 1986). Microepitope analysis has been reviewed.

Studies with Antipeptide Antibodies

In these studies, synthetic peptides are used to generate resulting antipeptide antibodies that react with the intact protein. A positive

tibodies may be facilitated by the peptide during formation (Getzoff et al., 1986; Getzoff et al., 1987), necessarily lead to a higher level (Jemmerson, 1987b; Wilson et al., 1988). A variety of strategies of conformational mimicry (Jemmerson, 1987c). Cyclization of the peptide (Jemmerson et al., 1971; Dorow et al., 1988; Jemmerson and Hutchinson, 1988) although it appears that information about the epitope is required to achieve this (Geysen et al., 1990). Other strategies for steric hindrance have also been proposed (Jemmerson et al., 1989).

These may be tested by measuring the antigenicity of the protein with its homologous antibodies on a solid phase and measuring the binding of antibodies. In the latter case, it is necessary to determine the test (Norrbom et al., 1988) and to establish the specific nature of the epitopes in which the liquid-phase binds the antigen and to show that the antigen is immunogenic (Jemmerson, 1987a). However, the antigen reacts with the antibody on can't be observed at low concentrations (Norrbom and Schechter, 1981).

thesis

without prior cleavage from the protein (Stanley, 1977; Shi et al., 1984). The method (Geysen et al., 1984, 1987b) allows the use of polyethylene pins. The pins are arranged in a grid format and spacing of a mixed by an enzyme immunoassay. In each test the pins can be freed with different antibody preparations. This method is ideally suited for the peptides of a protein, starting with the terminal one. Usually 6 to 10 peptides are used (Geysen et al., 1987b). The support may, in some cases,

impair its antigenic activity and it is thus possible to miss certain peptides that would be revealed as antigenically active in a different type of immunoassay. Another problem encountered with the pepscan technique is that some peptide sequences tend to give rise to nonspecific binding. The high concentration of peptide on the pins favors bivalent binding of antibody and facilitates the detection of very low levels of cross-reactivity. As a result, di- or tripeptide sequences may give rise to observable cross-reactions in this technique (Geysen et al., 1986; Trifilieff et al., 1991).

Identification of Critical Residues by Replacement Studies

The pepscan technique is also frequently used to determine the contribution of individual amino acids to the binding interaction between peptide and antibody (Getzoff et al., 1988). This is achieved by analyzing peptide replacement sets in which each residue of the peptide is, in turn, replaced by the other 19 possible amino acids. In this way some residues are found to be essential for binding since they cannot be replaced by any residue without impairing the antigenic reactivity. Presumably they correspond to critical residues that contribute to the energy of interaction. Other residues can be replaced by all common amino acids without affecting binding and their role may be limited to that of a scaffold. By analyzing the pattern of replaceability linked to retention of antibody binding in 103 continuous epitopes of various proteins, Geysen et al. (1988) found that, on average, only five out of six residues in hexapeptides were essential for activity.

Studies with Fusion Proteins and Peptides

Epitopes can be identified by expressing parts of the protein in a prokaryotic expression system and measuring the antigenic activity of the expression product (Lenstra et al., 1990). For instance, in the pEX expression system, the product of an inserted DNA fragment becomes the C-terminal portion of a hybrid galactosidase protein which precipitates in the cell. The hybrid protein is solubilized in sodium dodecyl sulfate (SDS) and transferred to a nitrocellulose filter for testing its antigenicity (Stanley, 1983). In recent years a number of expression vector systems have been developed (Charbit, 1991; Mehra et al., 1986; Young and Davis, 1983). Viral chimeras, in which the epitopes of one virus are inserted by recombinant DNA techniques into the particles of another virus, are also increasingly used (Clarke et al., 1987; Delpéroux et al., 1986; Michel et al., 1988). The recombinant approach to epitope analysis has been reviewed by Hofnung and Charbit (1992).

Studies with Antipeptide Antibodies

In these studies, synthetic peptides are used for immunization and the resulting antipeptide antibodies are tested for their ability to cross-react with the intact protein. A positive cross-reaction is taken as an indication that the

peptide approximates to an epitope of the protein. Although it has repeatedly been claimed (Green et al., 1982; Lern et al., 1984; Niman et al., 1983) that immunization with peptides leads to a very high frequency of induction of antibodies able to recognize the *native* protein, it is now generally accepted that these claims arose because the antipeptide antibodies reacted with denatured protein molecules present in solid-phase immunoassay (Jemmerson, 1987a; Jemmerson and Blankenfeld, 1989; Van Regenmortel, 1989a). It is now widely recognized that proteins become at least partly denatured when they are adsorbed to plastic during a solid-phase assay (Darst et al., 1988; Friguet et al., 1984; Soderquist et al., 1980) which explains why antipeptide antibodies frequently react quite well with plate-bound protein antigens (see Chapter 11).

The contention that antibodies against a highly disordered state (the peptide) are mostly able to recognize the highly ordered state (the folded protein in its native conformation), although the reverse is not necessarily the case, has been called the order-disorder paradox (Dyson et al., 1988). In an attempt to resolve this paradox, it was suggested that a preferred conformation of the peptide present in solution becomes stabilized at the surface of the carrier protein or when the peptide binds to the B-cell receptor during immune stimulation. However, if a process of induced fit is able to influence the conformation of the immunogenic form of the peptide, one would expect that a similar induction of conformation would occur when the peptide interacts with antiprotein antibodies. In other words, antibodies against the ordered state should also be able to recognize at high frequency the disordered peptide, which was in fact not observed (Green et al., 1982). The paradox simply vanishes, however, if it is accepted that the extent of cross-reactivity between peptides and native protein is always rather low, irrespective of whether antiprotein or antipeptide antibodies are tested. This low level of cross-reactivity is illustrated by the finding that intact cytochrome c was able to activate only a small fraction of peptide-primed B lymphocytes (Jemmerson and Blankenfeld, 1989).

In order to produce antipeptide antisera, it is customary to couple peptides of less than 10 to 15 residues to a carrier protein (Briand et al., 1985; Palfreyman et al., 1984; Van Regenmortel et al., 1988). Since the method of conjugation may strongly influence the type of antipeptide antibodies that are produced (Bahaoui et al., 1987; Dyrberg and Oldstone, 1986; Mariani et al., 1987; Schaaper et al., 1989), it is advisable to use more than one conjugation procedure and immunization schedule. An approach which avoids the formation of antibodies to a carrier protein is the multiple-antigen peptide (MAP) system introduced by Tam (1988). The MAP consists of a core matrix made up of three levels of lysine residues and eight amino terminals for anchoring peptide antigens. Although the MAP system enhances the immunogenicity of peptides and leads to high levels of peptide antibodies in immunized animals, the antibodies do not always cross-react strongly with the cognate protein.

Studies with Mutants and Site-Directed Mutagenesis

In this method the anti-presenting known amino acid substitution leads to a change in the mutated residue is involved (Wilson, 1984). In general, it is a protein only cause a local (Benjamin et al., 1984). The MAbs directed against neighbor the one substitution. There may alter the antigenicity (Moudallal et al., 1982; Barnet et al., 1988; Hurrell et al., 1988).

Since the number of available proteins is always limited, a protein variants by site-directed mutagenesis (Oertle et al., 1989) or by site-directed mutagenesis has the advantage of examining for any given amino acid contact residue if the substitution significantly increase the side-chain reactivity. A precondition is introduced that by a bulky side chain could be causing normal bond distance.

Instead of inferring the distinction between protein agents to select nonneutralizing pressure of neutralizing MAbs, the presence of neutralizing then sequenced to identify neutralization-resistant (Air and

Topographic Epitope Mapping

Competitive bindings as the relative position of epitopes. However, two epitopes are not far apart to allow simultaneous binding of antibody molecules, consisting of antibody fragments directed against distinct but overlapping epitopes. From binding simultaneously, each antibody fragment covers about not mean that the epitope necessarily covers all in proteins (Barlow et al., 1988).

in. Although it has repeatedly (Niman et al., 1983) that frequency of induction of it is now generally accepted that antibodies reacted with dense immunoassay (Jemmerson, in Regenmortel, 1989a). It is at least partly denatured when assay (Darst et al., 1988; which explains why antipeptide -bound protein antigens (see

highly disordered state (the powdered state (the folded protein is not necessarily the case, on et al., 1988). In an attempt to find conformation of the at the surface of the carrier cell receptor during immune fit is able to influence the peptide, one would expect that when the peptide interacts with antibodies against the ordered conformation the disordered peptide, 1982). The paradox simply that of cross-reactivity between low, irrespective of whether This low level of cross-reactivity was able to activate lymphocytes (Jemmerson and Blan-

is customary to couple peptides to protein (Briand et al., 1985; al., 1988). Since the method of antipeptide antibodies that and Oldstone, 1986; Mariani is possible to use more than one site. An approach which avoids the multiple-antigen peptide MAP consists of a core matrix and eight amino terminals for P system enhances the immunogenicity of peptide antibodies in immunological assays which cross-react strongly with the

Studies with Mutants and Analogs

In this method the antigenic cross-reactivity between related proteins presenting known amino acid substitutions is studied by means of MAbs. If the substitution leads to a change in antibody binding, it is assumed that the mutated residue is involved in the structure of an epitope (Hornbeck and Wilson, 1984). In general, it seems that single substitutions at the surface of a protein only cause a local change with no long-range structural alterations (Benjamin et al., 1984). This is demonstrated by the fact that usually other MAbs directed against neighboring epitopes of the protein are unaffected by the one substitution. There are exceptions, however, and certain mutations may alter the antigenicity by an indirect distal conformational effect (Al Moudallal et al., 1982; Barnett et al., 1989; Blondel et al., 1986; Collawn et al., 1988; Hurrell et al., 1977).

Since the number of available substitutions in a series of homologous proteins is always limited, a more extensive analysis may require generating protein variants by site-directed chemical modification (Cooper et al., 1987; Oertle et al., 1989) or by site-directed mutagenesis (Smith et al., 1991). This latter approach has the added advantage that multiple substitutions can be examined for any given amino acid. In this case, a residue is defined as a contact residue if the substitution leading to decreased binding does not significantly increase the side-chain volume (Smith and Benjamin, 1991). Such a precondition is introduced because substitution of a small noncontact residue by a bulky side chain could alter binding by preventing antibody from establishing normal bond distances with other contact residues (Fig. 2C).

Instead of inferring the position of epitopes from the ability of MAbs to distinguish between protein variants, it is possible in the case of infectious agents to select nonneutralizable mutants by the immunological selection pressure of neutralizing MAbs (Pollock et al., 1984). By growing a virus in the presence of neutralizing MAbs, escape mutants can be selected that are then sequenced to identify the substitution which rendered the mutant neutralization-resistant (Air and Laver, 1986; Wiley and Skehel, 1987).

Topographic Epitope Mapping

Competitive binding assays with pairs of MAbs can be used to determine the relative position of epitopes on the surface of a protein (Berzofsky, 1984). However, two epitopes are recognized as different only if they are far enough apart to allow simultaneous binding of the two MAbs. Because of the bulkiness of antibody molecules, considerable steric hindrance may occur and MAbs directed against distinct but neighboring epitopes could thus be presented from binding simultaneously to the antigen surface. Although a single Fab antibody fragment covers about 700 \AA^2 of the surface of an antigen, this does not mean that the epitope necessarily extends over the same area. Incidentally, this makes the argument for the exclusive existence of discontinuous epitopes in proteins (Barlow et al., 1986) less compelling, since a MAb recognizing,

for instance, a continuous epitope of only three residues would still cover an area of about 700 \AA^2 of the antigen surface.

When the number of MAbs used in epitope mapping is large, one often observes a continuum of epitopes that can no longer be subdivided into discrete, separate antigenic domains (Mathews and Roehrig, 1984; Underwood, 1982). Instead of blocking binding, a competing antibody may actually enhance the binding of a second antibody presumably by an allosteric effect (Cecilia et al., 1988; Heinz et al., 1984). Such enhancement of binding may be caused by only one of two antibodies (unidirectional enhancement) or it may be bidirectional (Heinz, 1986).

THE PREDICTION OF CONTINUOUS EPITOPES

Many attempts have been made to correlate the location of continuous epitopes in a few well-characterized proteins with parameters such as the hydrophilicity, accessibility, and mobility of short segments of their polypeptide chains. All prediction calculations are based on propensity scales for each of the 20 amino acids. These scales describe the tendency of each residue to be associated with properties such as surface accessibility or hydrophilicity. Usually a window of seven residues is used in the analysis. The corresponding value of the scales is introduced for each of the seven residues and the arithmetical mean of the seven values assigned to the center of the window.

Various algorithms for predicting secondary structure have also been applied to the prediction of continuous epitopes. Whereas the core of proteins usually contains a combination of helices and sheets, their surface is replete with turns and loops (Rose et al., 1985a). The success rate of secondary structure prediction algorithms is limited since at most 55 to 70% of the structural elements are correctly predicted (Fasman, 1989; Kabsch and Sander, 1983). The predictive value of eight scales has been compared, using as a criterion of success the number of residues correctly predicted to be antigenic in four well-studied proteins (Van Regenmortel and Daney de Marcillac, 1988). It was found that none of the methods achieved a high level of correct prediction, although the hydrophilicity scale of Parker et al. (1986) and the segmental mobility scale of Karplus and Schulz (1985) were slightly more successful than the others (Fig. 3).

In a recent study, the validity of 22 different scales for predicting antigenicity was analyzed using 9 proteins containing 54 identified continuous epitopes (Pellequer et al., 1991). The method of analysis calculated how many residues of each protein were correctly or wrongly predicted to be antigenic. The results obtained with 10 of the scales are summarized in Table 2. From the ratio of correctly over wrongly predicted residues it was found that various hydrophobicity and hydrophilicity scales gave 51 to 57% correct predictions. The accessibility scales gave 46 to 52% correct predictions, whereas the scales that predict turns gave a slightly higher level of correct prediction (53 to

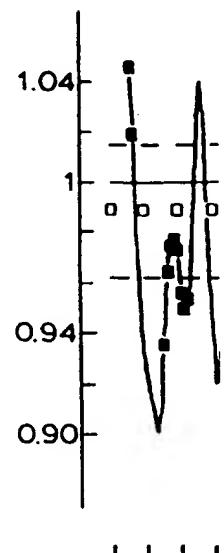


Figure 3. Prediction of bility profile of myoglob (1985). The smoothing p cillac (1988a) was used. are part of known conti represent the average va quence and the two hori mean. Such an interval

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CONCLUSION

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CONTINUOUS EPITOPES

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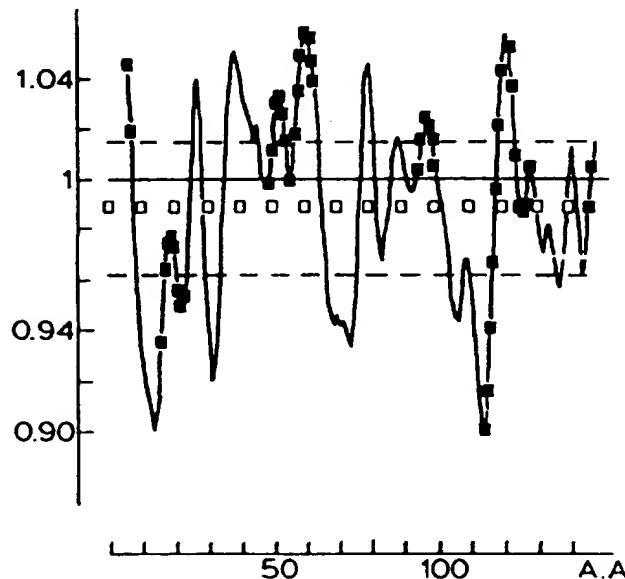


Figure 3. Prediction of continuous epitopes in proteins. Segmental mobility profile of myoglobin calculated with the scale of Karplus and Schulz (1985). The smoothing procedure of Van Regenmortel and Daney de Marcillac (1988a) was used. The black squares correspond to amino acids that are part of known continuous epitopes of myoglobin. The white squares represent the average value of the mobility parameter over the entire sequence and the two horizontal broken lines represent ± 0.7 SD from the mean. Such an interval corresponds to 50% of the population.

61%). These results again confirm earlier studies (Getzoff et al., 1988; Van Regenmortel and Daney de Marcillac, 1988) which showed that none of the algorithms in current use give a high level of correct prediction.

CONCLUSION

The different analytical approaches used to delineate protein epitopes lead to different perceptions of the nature of protein antigenicity. The structural approach of X-ray crystallography concentrates on the relative position of atoms at the antigen-antibody interface and identifies epitopes as structural entities of 15 to 22 residues. In contrast, the functional approach based on cross-reactive binding measurements introduces the additional dimension of time and leads to the view that only about five residues are implicated in epitopes defined in a functional way. Furthermore, binding measurements are unavoidably submitted to operational constraints and different immunoassays or different types of probes (for instance, free peptide, conjugated peptide, or solid-phase peptide) point to different residues as being critical in an

TABLE 2.
Comparative Value of 10 Antigenicity Prediction Scales Applied to Nine Proteins of Known Antigenic Structure

	Correctly Predicted Residues ^a	Wrongly Predicted Residues ^a	Ratio ^b	Percentage Correct Prediction ^c
Inverted hydrophobicity scale of Kyte and Doolittle, 1982	180	171	1.05	51
Inverted hydrophobicity scale of Rose et al., 1985b	150	134	1.11	53
Hydrophilicity scale of Hopp and Woods, 1981	150	147	1.02	51
Hydrophilicity scale of Parker et al., 1986	190	145	1.31	57
Accessibility scale of Chotia, 1976	180	167	1.07	52
Acrophilicity scale of Hopp, 1984	142	124	1.14	53
Flexibility scale of Karplus and Schulz, 1985	152	138	1.10	52
Antigenicity scale of Welling et al., 1985	133	236	0.56	36
Scale for turns of Chou and Fasman, 1978	151	111	1.36	58
Scale for turns of Levitt, 1976	169	108	1.56	61

^a The columns correctly predicted and wrongly predicted correspond to the number of correctly predicted and wrongly predicted amino acids, respectively, above the cut-off level ($+ 0.7 \times SE$).

^b Ratio of correctly predicted/wrongly predicted amino acids.

^c Ratio [correctly predicted/(correctly predicted + wrongly predicted)] expressed as percentage correct prediction.

Adapted from Pellequer et al., 1991.

epitope. Structural and functional analyses have been termed "two different ways of seeing" (Lambert and Hughes, 1988), and these two approaches lead to complementary models of biological and immunological reality.

Epitopes are relational entities since they can be recognized only by the binding of complementary paratopes. An epitope is thus not an intrinsic feature of a protein molecule existing independently of its paratope partner. Epitopes and antigens can be defined only in terms of the emerging properties and relationships that arise in an immunological system.

The molecular dissection of protein antigens should thus not be confused with an attempt to reduce biology to chemistry. The distinction between an immunochemical interaction and a simple chemical one remains valid also when the binding reaction between two immunological reactants is analyzed in molecular terms.

REFERENCES

- Absalom, D., and Van Regenmortel, M. H. V. (1985) Immunochemical absorption and immunochemical analysis of antibodies against influenza virus. *Adv. Vir. Res.* 35:1005—1010.
- Al Moudallal, Z., Briand, J. P., and Schubert, C. (1988) Synthetic peptides as probes of the antigenic sites of the hepatitis B virus surface antigen. *J. Virol.* 52:1231—1235.
- Altschuh, D., and Van Regenmortel, M. H. V. (1986) Determinants of a viral protein antigenic site: identification of the major determinants of a viral protein antigen by immunological mapping with tryptic peptides. *Proc. Natl. Acad. Sci. U.S.A.* 83:747—753.
- Anderer, F. A., and Schlumberger, M. (1985) Immunological relationship between terminal amino acid and dipeptide sequence. *Acta Virol.* 31:222—224.
- Aron, R., Maron, E., Sela, M., and Shilo, B. (1985) Immunological studies with native lysozyme elicited in mice. *Proc. Natl. Acad. Sci. U.S.A.* 82:1450—1455.
- Atassi, M. Z. (1984) Antigenic sites in proteins. In: *Antigenic sites in peptides and proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Atherton, E., and Sheppard, R. C. (1988) *Antigenic Site Approach*. IRL Press, Oxford.
- Bahraoui, E., El Ayeb, M., Grainger, R., and Gheorghiu, L. (1988) Immunological properties of scorpion toxins. Immunogenicity and relatively rigid region. *Eur. J. Biochem.* 183:101—106.
- Barlow, D. J., Edwards, M. S., and Hart, D. J. (1988) Continuous protein antigenic determinants. *Europ. J. Biochem.* 183:107—112.
- Barnett, P. V., Ouldridge, E. J., and Hart, D. J. (1989) Neutralizing epitopes of hepatitis B virus and characterization of three functional epitopes. *J. Gen. Virol.* 70:1483—1491.
- Benjamin, D. C., Berzofsky, J. A., Margoliash, E., Michaelson, J., Sercarz, E. E., Smith-Gill, S. J., and Tamm, I. H. (1984) Antigenic structure of proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (1977) *Immunochemistry of Proteins*, Vol. 1. Academic Press, London.
- Benjamini, E. (1984) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (1985) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (1986) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (1987) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (1988) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (1989) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (1990) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (1991) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (1992) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (1993) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (1994) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (1995) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (1996) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (1997) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (1998) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (1999) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2000) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2001) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2002) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2003) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2004) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2005) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2006) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2007) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2008) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2009) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2010) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2011) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2012) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2013) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2014) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2015) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2016) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2017) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2018) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2019) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2020) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2021) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2022) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2023) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2024) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2025) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2026) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2027) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2028) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2029) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2030) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2031) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2032) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2033) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2034) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2035) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2036) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2037) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2038) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2039) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2040) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2041) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2042) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2043) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2044) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2045) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2046) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2047) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2048) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2049) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.
- Benjamini, E. (2050) Antigenic sites in proteins. In: *Antigenic Sites in Peptides and Proteins*. Atassi, M. Z., and Smith, J. A., eds. Academic Press, London.

**Prediction Scales Applied
to Antigenic Structure**

Wrongly predicted amino acid residues ^a	Ratio ^b	Percentage Correct Prediction ^c
171	1.05	51
34	1.11	53
47	1.02	51
45	1.31	57
67	1.07	52
24	1.14	53
38	1.10	52
36	0.56	36
11	1.36	58
08	1.56	61

^apredicted correspond to the number of acids, respectively, above the cut-off

^bno acids.
wrongly predicted)] expressed as

ve been termed "two different", and these two approaches lead immunological reality.

can be recognized only by the peptide is thus not an intrinsic feature of its paratope partner. Epitopes of the emerging properties and system.

ns should thus not be confused by. The distinction between an emical one remains valid also biological reactants is analyzed

REFERENCES

- Absalom, D., and Van Regenmortel, M. H. V. (1977) Purification by immunoabsorption and immunochemical properties of histone H3. *FEBS Lett.* 81:419—422.
- Air, G. M., and Laver, W. G. (1986) The molecular basis of antigenic variation in influenza virus. *Adv. Vir. Res.* 31:53—102.
- Al Moudallal, Z., Briand, J. P., and Van Regenmortel, M. H. V. (1982) Monoclonal antibodies as probes of the antigenic structure of tobacco mosaic virus. *EMBO J.* 1:1005—1010.
- Al Moudallal, Z., Briand, J. P., and Van Regenmortel, M. H. V. (1985) A major part of the polypeptide chain of tobacco mosaic virus protein is antigenic. *EMBO J.* 4:1231—1235.
- Altschuh, D., and Van Regenmortel, M. H. V. (1982) Localization of antigenic determinants of a viral protein by inhibition of enzyme-linked immunosorbent assay (ELISA) with tryptic peptides. *J. Immunol. Meth.* 50:99—108.
- Amit, A. G., Mariuzza, R. A., Phillips, S. E. V., and Poljak, R. J. (1986) Three-dimensional structure of an antigen-antibody complex at 2.8 Å resolution. *Science* 233:747—753.
- Anderer, F. A., and Schlumberger, H. D. (1965) Properties of different artificial antigens immunologically related to tobacco mosaic virus. *Biochim. Biophys. Acta* 97:503—509.
- Anderer, F. A., and Schlumberger, H. D. (1966) Cross-reactions of antisera against the terminal amino acid and dipeptide of tobacco mosaic virus. *Biochim. Biophys. Acta* 115:222—224.
- Arnon, R., Maron, E., Sela, M., and Anfinsen, C. B. (1971) Antibodies reactive with native lysozyme elicited by a completely synthetic antigen. *Proc. Natl. Acad. Sci. U.S.A.* 68:1450—1455.
- Atassi, M. Z. (1984) Antigenic structures of proteins. Their determination has revealed important aspects of immune recognition and generated strategies for synthetic mimicking of protein binding sites. *Eur. J. Biochem.* 145:1—20.
- Atassi, M. Z., and Smith, J. A. (1978) A proposal for the nomenclature of antigenic sites in peptides and proteins. *Immunochemistry* 15:609—610.
- Atherton, E., and Sheppard, R. C. (1989) *Solid Phase Peptide Synthesis. A Practical Approach*. IRL Press, Oxford, 203.
- Bahraoui, E., El Ayeb, M., Granier, C., and Rochat, H. (1987) Immunochemistry of scorpion toxins. Immunogenicity of peptide 19—28 a model of an accessible and relatively rigid region. *Eur. J. Biochem.* 167:371—375.
- Barlow, D. J., Edwards, M. S., and Thornton, J. M. (1986) Continuous and discontinuous protein antigenic determinants. *Nature (London)*, 322:747—748.
- Barnett, P. V., Ouldridge, E. J., Rowlands, D. J., Brown, F., and Parry, N. R. (1989) Neutralizing epitopes of type O foot-and-mouth disease virus. I. Identification and characterization of three functionally independent, conformational sites. *J. Gen. Virol.* 70:1483—1491.
- Benjamin, D. C., Berzofsky, J. A., East, I. J., Gurd, F. R. N., Hannum, C., Leach, S. J., Margoliash, E., Michael, J. G., Miller, A., Prager, E. M., Reichlin, M., Sercarz, E. E., Smith-Gill, S. J., Todd, P. E., and Wilson, A. C. (1984) The antigenic structure of proteins: a reappraisal. *Annu. Rev. Immunol.* 2:67—101.
- Benjamini, E. (1977) Immunochemistry of the tobacco mosaic virus protein. In *Immunochemistry of Proteins*, Vol. 2 (M. Z. Atassi, ed.), Plenum Press, NY, pp. 265—310.

- Berzofsky, J. A. (1984) Monoclonal antibodies as probes of antigenic structures. In *Monoclonal and Anti-idiotypic Antibodies: Probes for Receptor Structure and Function* (J. C. Venter, C. M. Fraser, and J. Lindstrom, eds.), Alan R. Liss, NY, pp. 1—19.
- Berzofsky, J. A. (1985) Intrinsic and extrinsic factors in protein antigenic structure. *Science* 229:932—940.
- Berzofsky, J. A., and Schechter, A. N. (1981) The concepts of crossreactivity and specificity in immunology. *Mol. Immunol.* 18:751—763.
- Bhat, T. N., Bentley, G. A., Fischmann, T. O., Boulot, G., and Poljak, R. J. (1990) Small rearrangements in structures of Fv and Fab fragments of antibody D1.3 on antigen binding. *Nature (London)* 347:483—485.
- Bittle, J. L., Houghten, R. A., Alexander, H., Shinnick, T., Sutcliffe, J. G., Lerner, R. A., Rowlands, D. J., and Brown, F. (1982) Protection against foot-and-mouth disease by immunization with a chemically synthesized peptide predicted from the viral nucleotide sequence. *Nature (London)* 298:30—33.
- Blondel, B., Crainic, R., Fichot, O., Dufraisse, G., Candrea, A., Diamond, D., Girard, M., and Horaud, F. (1986) Mutations conferring resistance to neutralization with monoclonal antibodies in type 1 poliovirus can be located outside or inside the antibody-binding site. *J. Virol.* 57:81—90.
- Briand, J. P., Al Moudallal, Z., and Van Regenmortel, M. H. V. (1982) Serological differentiation of tobamoviruses by means of monoclonal antibodies. *J. Virol. Meth.* 5:293—300.
- Briand, J. P., Muller, S., and Van Regenmortel, M. H. V. (1985) Synthetic peptides as antigens: pitfalls of conjugation methods. *J. Immunol. Meth.* 78:59—69.
- Cecilia, D., Gadkari, D. A., Kedarnath, N., and Ghosh, S. N. (1988) Epitope mapping of Japanese encephalitis virus envelope protein using monoclonal antibodies against an Indian strain. *J. Gen. Virol.* 69:2741—2747.
- Charbit, A. (1991) Expression de peptides étrangers sous forme de fusions génétiques de protéines chez les bactéries et les bactériophages. *Bull. Inst. Pasteur* 89:17—49.
- Chothia, C. (1976) The nature of the accessible and buried surfaces in proteins. *J. Mol. Biol.* 105:1—14.
- Chou, P. Y., and Fasman, G. D. (1978) Prediction of secondary structure of proteins from amino acid sequence. *Adv. Enzymol. Relat. Subj. Biochem.* 47:45—148.
- Clarke, B. E., Newton, S. E., Carroll, A. R., Francis, M. J., Appleyard, G., Syred, A. D., Highfield, P. E., Rowlands, D. J., and Brown, F. (1987) Improved immunogenicity of a peptide epitope after fusion to hepatitis B core protein. *Nature (London)* 330:381—384.
- Collawn, J. F., Wallace, C. J. A., Proudfoot, A. E. I., and Paterson, Y. (1988) Monoclonal antibodies as probes of conformational changes in protein-engineered cytochrome c. *J. Biol. Chem.* 263:8625—8634.
- Colman, P. M. (1988) Structure of antibody-antigen complexes: implications for immune recognition. *Adv. Immunol.* 43:99—132.
- Colman, P. M., Laver, W. G., Varghese, J. N., Baker, A. T., Tulloch, P. A., Air, G. M., and Webster, R. G. (1987) Three-dimensional structure of a complex of antibody with influenza virus neuraminidase. *Nature (London)* 326:358—363.
- Cooper, H. M., Jemmerson, R., Hunt, D. F., Griffin, R., Yates, J. R., Shabanowitz, J., Zhu, N. Z., and Paterson, Y. (1987) Site-directed chemical modification of horse cytochrome c results in changes in antigenicity due to local and long-range conformational perturbations. *J. Biol. Chem.* 262:11591—11597.
- Crumpton, M. J. (1986) The interaction of globular protein. *Symp.* 119:93—106.
- Darst, S. A., Robertson, C. R., and antigen myoglobin affects the bodies. *Biophys. J.* 53:533—539.
- Davies, D. R., Sheriff, S., and *J. Biol. Chem.* 263:10541—10545.
- Delpyroux, F., Chenciner, N., Van der Werf, S., and Streeel expressed on hybrid hepatitis 1
- DiMarchi, R., Brooke, G., Gale, Protection of cattle against foot-and-mouth disease by immunization with a synthetic peptide corresponding to the B-cell epitope of the B surface antigen after a single injection. *Nature (London)* 295:158—161.
- Dyrberg, T., and Oldstone, M. B. (1984) Hepatitis B virus-specific T-cell orientation. *J. Exp. Med.* 164:1732—1741.
- Dyson, H. J., Lerner, R. A., and of protein-reactive anti-peptides. *J. Immunol.* 137:305—324.
- Edmundson, A. B., Ely, K. R., and of opioid peptides to the Mcg 1 protein. *Mol. Immunol.* 24:915—935.
- Emini, E. A., Hughes, J. V., and hepatitis A virus-neutralizing antibodies. *J. Immunol.* 155:836—839.
- Fasman, G. D. (1989) *Predicting Protein Conformation*, Plenum Press, New York.
- Fieser, T. M., Tainer, J. A., and of protein flexibility and peptide antibodies with a 1991:84:8568—8572.
- Fourquet, P., Bahraoui, E., Forstgård, A., and Granier, C. (1988) Immunogenic properties of a modified peptide. *Peptide Protein Res.* 32:81—84.
- Friguet, B., Djavadi-Ohaniance, L., and antibodies raised with a native protein. *Mol. Immunol.* 21:673—677.
- Geerlings, H. J., Weijer, W. J., and S. (1988) The influence of p-herpes simplex virus type 1 in methionine. *J. Immunol.* 140:239—244.

- probes of antigenic structures. In *Topics in Receptor Structure and Function*, (eds.), Alan R. Liss, NY, pp. 139-155.
- tors in protein antigenic structure. The concepts of crossreactivity and specificity. *J. Immunol.* 131:751-763.
- Boulot, G., and Poljak, R. J. (1990) Identification of fragments of antibody D1.3 on the surface of the virus. *Nature (London)* 345:30-33.
- Snick, T., Sutcliffe, J. G., Lerner, R. A., and Poljak, R. J. (1989) Protection against foot-and-mouth disease by a synthetic peptide predicted from the structure of the virus. *Nature (London)* 339:30-33.
- G., Candrea, A., Diamond, D., and Poljak, R. J. (1989) Determinants of resistance to neutralization by monoclonal antibodies can be located outside or inside the virus capsid. *Nature (London)* 339:33-36.
- Ortel, M. H. V. (1982) Serological responses to synthetic peptides. *J. Virol. Meth.* 18:1-10.
- I. H. V. (1985) Synthetic peptides as immunogens. *J. Virol. Meth.* 18:59-69.
- Wosh, S. N. (1988) Epitope mapping using monoclonal antibodies against hepatitis B surface antigen. *Nature (London)* 332:17-19.
- s sous forme de fusions génétiques et leur expression. *Bull. Inst. Pasteur* 89:17-49.
- and buried surfaces in proteins. *J. Mol. Biol.* 205:145-158.
- of secondary structure of proteins and their interaction with antibodies. *Subj. Biochem.* 47:45-148.
- McLachlan, M. J., Appleyard, G., Syred, S., and Brown, F. (1987) Improved immunogenicity of the hepatitis B core protein. *Nature (London)* 326:358-363.
- .. E. I., and Paterson, Y. (1988) Changes in protein-engineered antigen complexes: implications for antibody recognition. *J. Mol. Biol.* 205:145-158.
- Baker, A. T., Tulloch, P. A., Air, G., and Strelakovsky, A. (1988) The three-dimensional structure of a complex of antibodies and antigen. *Nature (London)* 332:17-49.
- in, R., Yates, J. R., Shabanowitz, J., and Hunt, D. F. (1988) Directed chemical modification of antigenic sites due to local and long-range interactions. *J. Mol. Biol.* 212:11591-11597.
- Crumpton, M. J. (1986) The importance of conformation and of equilibria in the interaction of globular proteins and their fragments with antibodies. *Ciba Found. Symp.* 119:93-106.
- Darst, S. A., Robertson, C. R., and Berzofsky, J. A. (1988) Adsorption of the protein antigen myoglobin affects the binding of conformation-specific monoclonal antibodies. *Biophys. J.* 53:533-539.
- Davies, D. R., Sheriff, S., and Padlan, E. A. (1988) Antigen-antibody complexes. *J. Biol. Chem.* 263:10541-10544.
- Delpéroux, F., Chenciner, N., Lim, A., Malpiece, Y., Blondel, B., Crainic, R., Van der Werf, S., and Strecker, R. E. (1986) A poliovirus neutralization epitope expressed on hybrid hepatitis B surface antigen particles. *Science* 233:472-475.
- DiMarchi, R., Brooke, G., Gale, C., Cracknell, V., Doel, T., and Mowat, N. (1986) Protection of cattle against foot-and-mouth disease by a synthetic peptide. *Science* 232:639-641.
- Dorow, D. S., Shi, P. T., Carbone, F. R., Minasian, E., Todd, P. E. E., and Leach, S. J. (1985) Two large immunogenic and antigenic myoglobin peptides and the effects of cyclisation. *Mol. Immunol.* 22:1255-1264.
- Dreesman, G. R., Sanchez, Y., Ionescu-Matiu, I., Sparrow, J. T., Six, H. R., Peterson, D. L., Hollinger, F. B., and Melnick, J. L. (1982) Antibody to hepatitis B surface antigen after a single inoculation of uncoupled synthetic HBsAg peptides. *Nature (London)* 295:158-160.
- Dyrberg, T., and Oldstone, M. B. A. (1986) Peptides as antigens. Importance of orientation. *J. Exp. Med.* 164:1344-1349.
- Dyson, H. J., Lerner, R. A., and Wright, P. E. (1988) The physical basis for induction of protein-reactive anti-peptide antibodies. *Ann. Rev. Biophys. Biophys. Chem.* 17:305-324.
- Edmundson, A. B., Ely, K. R., Herron, J. N., and Cheson, B. D. (1987) The binding of opioid peptides to the Mcg light chain dimer: flexible keys and adjustable locks. *Mol. Immunol.* 24:915-935.
- Emini, E. A., Hughes, J. V., Perlow, D. S., and Boger, J. (1985) Induction of hepatitis A virus-neutralizing antibody by a virus-specific synthetic peptide. *J. Virol.* 55:836-839.
- Fasman, G. D. (1989) *Prediction of Protein Structure and the Principles of Protein Conformation*, Plenum Press, NY.
- Fieser, T. M., Tainer, J. A., Geysen, H. M., and Houghten, R. A. (1987) Influence of protein flexibility and peptide conformation on reactivity of monoclonal anti-peptide antibodies with a protein α -helix. *Proc. Natl. Acad. Sci. U.S.A.* 84:8568-8572.
- Fourquet, P., Bahraoui, E., Fontecilla-Camps, J. C., Van Rietschoten, J., Rochat, H., and Granier, C. (1988) Immunochemistry of scorpion toxins. Synthesis and antigenic properties of a model of a loop region specific to alpha-toxins. *Int. J. Peptide Protein Res.* 32:81-88.
- Friguet, B., Djavadi-Ohaniance, L., and Goldberg, M. E. (1984) Some monoclonal antibodies raised with a native protein bind preferentially to the denatured antigen. *Mol. Immunol.* 21:673-677.
- Geerlings, H. J., Weijer, W. J., Bloemhoff, W., Welling, G. W., and Welling-Wester, S. (1988) The influence of pH and ionic strength on the coating of peptides of herpes simplex virus type 1 in an enzyme-linked immunosorbent assay. *J. Immunol. Meth.* 106:239-244.

- Getzoff, E. D., Geysen, H. M., Rodda, S. J., Alexander, H., Tainer, J. A., and Lerner, R. A. (1987) Mechanisms of antibody binding to a protein. *Science* 235:1191—1196.
- Getzoff, E. D., Tainer, J. A., Lerner, R. A., and Geysen, H. M. (1988) The chemistry and mechanism of antibody binding to protein antigens. *Adv. Immunol.* 43:1—98.
- Geysen, H. M. (1985) Antigen-antibody interactions at the molecular level: adventures in peptide synthesis. *Immunol. Today* 6:364—369.
- Geysen, H. M., Mason, T. J., and Rodda, S. J. (1988) Cognitive features of continuous antigenic determinants. *J. Mol. Recognition* 1:32—41.
- Geysen, H. M., Meloen, R. H., and Barteling, S. J. (1984) Use of peptide synthesis to probe viral antigens for epitopes to a resolution of a single amino acid. *Proc. Natl. Acad. Sci. U.S.A.* 81:3998—4002.
- Geysen, H. M., Rodda, S. J., and Mason, T. J. (1986) A priori delineation of a peptide which mimics a discontinuous antigenic determinant. *Mol. Immunol.* 23:709—715.
- Geysen, H. M., Rodda, S. J., Mason, T. J., Tainer, J. A., Alexander, H., Getzoff, E. D., and Lerner, R. A. (1987a) Antigenicity of myohemerythrin. *Science* 238:1584—1586.
- Geysen, H. M., Rodda, S. J., Mason, T. J., Tribbick, G., and Schoofs, P. G. (1987b) Strategies for epitope analysis using peptide synthesis. *J. Immunol. Meth.* 102:259—274.
- Ghosh, S., and Cambell, A. M. (1986) Multispecific monoclonal antibodies. *Immunol. Today* 7:217—222.
- Gras-Masse, H. S., Jolivet, M. E., Audibert, F. M., Beachey, E. H., Chedid, L. A., and Tartar, A. L. (1986) Influence of CONH₂ or COOH as C-terminus groups on the antigenic characters of immunogenic peptides. *Mol. Immunol.* 23:1391—1395.
- Gras-Masse, H., Jolivet, M., Drobecq, H., Aubert, J. P., Beachey, E. H., Audibert, F., Chedid, L., and Tartar, A. (1988) Influence of helical organization on immunogenicity and antigenicity of synthetic peptides. *Mol. Immunol.* 25:673—678.
- Green, N., Alexander, H., Olson, A., Alexander, S., Shinnick, T. M., Sutcliffe, J. G., and Lerner, R. A. (1982) Immunogenic structure of the influenza virus hemagglutinin. *Cell* 28:477—487.
- Harper, M., Lema, F., Boulot, G., and Poljak, R. J. (1987) Antigen specificity and cross-reactivity of monoclonal anti-lysozyme antibodies. *Mol. Immunol.* 24:97—108.
- Heinz, F. X. (1986) Epitope mapping of flavivirus glycoproteins. *Adv. Vir. Res.* 31:103—168.
- Heinz, F. X., Mandl, C., Berger, R., Tuma, W., and Kunz, C. (1984) Antibody-induced conformational changes result in enhanced avidity of antibodies to different antigenic sites on the tick-borne encephalitis virus glycoprotein. *Virology* 133:25—34.
- Hirayama, A., Takagaki, Y., and Karush, F. (1985) Interaction of monoclonal anti-peptide antibodies with lysozyme. *J. Immunol.* 134:3241—3247.
- Hodges, R. S., Heaton, R. J., and Parker, J. M. (1988) Antigen-antibody interaction. Synthetic peptides define linear antigenic determinants recognized by monoclonal antibodies directed to the cytoplasmic carboxyl terminus of rhodopsin. *J. Biol. Chem.* 263:11768—11775.
- Hofnung, M., and Charbit, A. (1992) Expression of antigens as recombinant proteins. In *Structure of Antigens* (M. H. V. Van Regenmortel ed.), Vol. 2, Telford Press, Boca Raton, FL, in press.
- Hopp, T. P. (1984) Protein and algorithms; selection of antigen.
- Hopp, T. P. (1986) Protein sur terminants and other interacti.
- Hopp, T. P., and Woods, K. R from amino acid sequence. *P*
- Hornbeck, P. V., and Wilson, A on the active site region of lys results. *Biochemistry* 23:998-
- Houghten, R. A. (1985) Genera numbers of peptides: specific individual amino acids. *Proc.*
- Hurrell, J. G. R., Smith, J. A., between mammalian myoglobin. *myochemistry* 14:283—288.
- Jemmerson, R. (1987a) Antigen frequency of peptide react 84:9180—9184.
- Jemmerson, R. (1987b) Polypep subset of secondary B lymphc 139:1939—1945.
- Jemmerson, R., and Blankenfel synthetic vaccines intended to
- Jemmerson, R., and Hutchinson. for synthetic epitopes by alter peptides. *Eur. J. Immunol.* 20
- Jemmerson, R., and Paterson, Y cations for the design of synt
- Kabsch, W., and Sander, C. (19 structure? *FEBS Lett.*, 155:1
- Karplus, P. A., and Schulz, G. *Naturwissenschaften* 72:212—
- Kent, S., and Clark-Lewis, I. (1 biologically active peptides.
- Alitalo, P., Partanen, and A.
- Kyte, J., and Doolittle, R. F. (19 character of a protein. *J. Mol*
- Lambert, D. M., and Hughes, , and functionalist biology. *J. ;*
- Lando, G., and Reichlin, M. (19 II. Characterization of antibo response to immunization wit
- Laver, W. G., Air, G. M., We on protein antigens: misconce
- Lenstra, J. A., Kusters, J. G., ai epitopes with prokaryotic exp
- Lerner, R. A. (1984) Antibodies *Adv. Immunol.* 36:1—44.
- Levitt, M. (1976) A simplified simulation of protein folding.

- Alexander, H., Tainer, J. A., and dy binding to a protein. *Science*
- leyzen, H. M. (1988) The chemistry of antigens. *Adv. Immunol.* 43:1—98.
- ns at the molecular level: adventures 369.
- 88) Cognitive features of continuous 32—41.
- J. (1984) Use of peptide synthesis 32—41.
- tion of a single amino acid. *Proc.*
- (1986) A priori delineation of a 32—41.
- enic determinant. *Mol. Immunol.*
- ter, J. A., Alexander, H., Getzoff, 32—41.
- icity of myohemerythrin. *Science*
- ick, G., and Schoofs, P. G. (1987b) 32—41.
- le synthesis. *J. Immunol. Meth.*
- ic monoclonal antibodies. *Immunol.*
- F. M., Beachey, E. H., Chedid, CONH₂ or COOH as C-terminus 32—41.
- nogenic peptides. *Mol. Immunol.*
- t, J. P., Beachey, E. H., Audibert, 32—41.
- ce of helical organization on imides. *Mol. Immunol.* 25:673—678.
- r, S., Shinnick, T. M., Sutcliffe, 32—41.
- ic structure of the influenza virus 32—41.
- .. J. (1987) Antigen specificity and 32—41.
- bodies. *Mol. Immunol.* 24:97—108.
- irus glycoproteins. *Adv. Vir. Res.*
- ., and Kunz, C. (1984) Antibody-mediated avidity of antibodies to different 32—41.
- glycoprotein. *Virology* 133:25—34.
- 85) Interaction of monoclonal anti- 32—41.
- 134:3241—3247.
- 1988) Antigen-antibody interaction. 32—41.
- minants recognized by monoclonal 32—41.
- /1 terminus of rhodopsin. *J. Biol.*
- of antigens as recombinant proteins. 32—41.
- morteil ed.), Vol. 2, Telford Press,
- Hopp, T. P. (1984) Protein antigen conformation: folding patterns and predictive algorithms; selection of antigenic and immunogenic peptides. *Ann. Sciavo* 2:47—60.
- Hopp, T. P. (1986) Protein surface analysis. Methods for identifying antigenic determinants and other interaction sites. *J. Immunol. Meth.* 88:1—18.
- Hopp, T. P., and Woods, K. R. (1981) Prediction of protein antigenic determinants from amino acid sequence. *Proc. Natl. Acad. Sci. U.S.A.* 78:3824—3828.
- Hornbeck, P. V., and Wilson, A. C. (1984) Local effects of amino acid substitutions on the active site region of lysozyme: a comparison of physical and immunological results. *Biochemistry* 23:998—1002.
- Houghten, R. A. (1985) General method for the rapid solid-phase synthesis of large numbers of peptides: specificity of antigen-antibody interaction at the level of individual amino acids. *Proc. Natl. Acad. Sci. U.S.A.* 82:5131—5135.
- Hurrell, J. G. R., Smith, J. A., Todd, P. E., and Leach, S. J. (1977) Cross-reactivity between mammalian myoglobins: linear vs. spacial antigenic determinants. *Immunochemistry* 14:283—288.
- Jemmerson, R. (1987a) Antigenicity and native structure of globular proteins: low frequency of peptide reactive antibodies. *Proc. Natl. Acad. Sci. U.S.A.* 84:9180—9184.
- Jemmerson, R. (1987b) Polypeptide fragments of horse cytochrome c activate a small subset of secondary B lymphocytes primed against the native protein. *J. Immunol.* 139:1939—1945.
- Jemmerson, R., and Blankenfeld, R. (1989) Affinity consideration in the design of synthetic vaccines intended to elicit antibodies. *Mol. Immunol.* 26:301—307.
- Jemmerson, R., and Hutchinson, R. M. (1990) Fine manipulation of antibody affinity for synthetic epitopes by altering peptide structure: antibody binding to looped peptides. *Eur. J. Immunol.* 20:579—585.
- Jemmerson, R., and Paterson, Y. (1986) Mapping antigenic sites on proteins: implications for the design of synthetic vaccines. *BioTechniques* 4:18—31.
- Kabsch, W., and Sander, C. (1983) How good are predictions of protein secondary structure? *FEBS Lett.*, 155:179—182.
- Karplus, P. A., and Schulz, G. E. (1985) Prediction of chain flexibility in proteins. *Naturwissenschaften* 72:212—213.
- Kent, S., and Clark-Lewis, I. (1985) Modern methods for the chemical synthesis of biologically active peptides. In *Synthetic Peptides in Biology and Medicine* (K. Alitalo, P. Partanen, and A. Vaheri, eds.), Elsevier, NY, pp. 29—57.
- Kyte, J., and Doolittle, R. F. (1982) A simple method for displaying the hydrophobic character of a protein. *J. Mol. Biol.* 157:105—132.
- Lambert, D. M., and Hughes, A. J. (1988) Keywords and concepts in structuralist and functionalist biology. *J. Theor. Biol.* 133:133—145.
- Lando, G., and Reichlin, M. (1982) Antigenic structure of sperm whale myoglobin. II. Characterization of antibodies preferentially reactive with peptides arising in response to immunization with the native protein. *J. Immunol.* 129:212—216.
- Laver, W. G., Air, G. M., Webster, R. G., and Smith-Gill, S. J. (1990) Epitopes on protein antigens: misconceptions and realities. *Cell* 61:553—556.
- Lenstra, J. A., Kusters, J. G., and Van der Zeijst, B. A. M. (1990) Mapping of viral epitopes with prokaryotic expression products. *Arch. Virol.* 110:1—24.
- Lerner, R. A. (1984) Antibodies of predetermined specificity in biology and medicine. *Adv. Immunol.* 36:1—44.
- Levitt, M. (1976) A simplified representation of protein conformations for rapid simulation of protein folding. *J. Mol. Biol.* 104:59—107.

- Mäkelä, O. (1965) Single lymph node cells producing heteroclitic bacteriophage antibody. *J. Immunol.* 95:378—386.
- Mariani, M., Bracci, L., Presentini, R., Nucci, D., Neri, P., and Antoni, G. (1987) Immunogenicity of a free synthetic peptide: carrier-conjugation enhances antibody affinity for the native protein. *Mol. Immunol.* 24:297—303.
- Mariuzza, R. A., Phillips, S. E. V., and Poljack, R. J. (1987) The structural basis of antigen-antibody recognition. *Annu. Rev. Biophys. Chem.* 16:139—159.
- Mathews, J. H., and Roehrig, J. T. (1984) Elucidation of the topography and determination of the protective epitopes on the E glycoprotein of Saint Louis encephalitis virus by passive transfer with monoclonal antibodies. *J. Immunol.* 132:1533—1537.
- McCray, J., and Werner, G. (1987) Different rhinovirus serotypes neutralized by antipeptide antibodies. *Nature (London)* 329:736—738.
- Mehra, V., Sweetser, D., and Young, R. A. (1986) Efficient mapping of protein antigenic determinants. *Proc. Natl. Acad. Sci. U.S.A.* 83:7013-7017.
- Michel, M. L., Mancini, M., Sobczak, E., Favier, V., Guetard, D., Bahraoui, E. M., and Tiollais, P. (1988) Induction of anti-human immunodeficiency virus (HIV) neutralizing antibodies in rabbits immunized with recombinant HIV-hepatitis B surface antigen particles. *Proc. Natl. Acad. Sci. U.S.A.* 85:7957—7961.
- Milton De, L. R. C., and Van Regenmortel, M. H. V. (1979) Immunochemical studies of tobacco mosaic virus. III. Demonstration of five antigenic regions in the protein subunit. *Mol. Immunol.* 16:179—184.
- Muller, S., Plaué, S., Couppez, M., and Van Regenmortel, M. H. V. (1986) Comparison of different methods for localizing antigenic regions in histone H2A. *Mol. Immunol.* 23:593—601.
- Muller, S., Plaué, S., Samama, J. P., Valette, M., Briand, J. P., and Van Regenmortel, M. H. V. (1990) Antigenic properties and protective capacity of a cyclic peptide corresponding to site A of influenza virus haemagglutinin. *Vaccine* 8:308—314.
- Mutter, M. (1988) Nature's rules and chemist's tools: a way for creating novel proteins. *Trends Biochem. Sci.* 13:260—265.
- Nestorowicz, A., Tregear, G. W., Southwell, C. N., Martyn, J., Murray, J. M., White, D. O., and Jackson, D. J. (1985) Antibodies elicited by influenza virus hemagglutinin fail to bind to synthetic peptides representing putative antigenic sites. *Mol. Immunol.* 22:145—154.
- Niman, H. L., Houghten, R. A., Walker, L. A., Reisfeld, R. A., Wilson, I. A., Hogle, J. M., and Lerner, R. A. (1983) Generation of protein-reactive antibodies by short peptides is an event of high frequency: implications for the structural basis of immune recognition. *Proc. Natl. Acad. Sci. U.S.A.* 80:4949—4953.
- Norrby, E., Mufson, M. A., Alexander, H., Houghten, R. A., and Lerner, R. A. (1987) Site-directed serology with synthetic peptides representing the large glycoprotein G of respiratory syncytial virus. *Proc. Natl. Acad. Sci. U.S.A.* 84:6572—6576.
- Novotny, J., Brucolieri, R. E., Carlson, W. D., Handschumacher, M., and Haber, E. (1987b) Antigenicity of myohemerythrin. *Science* 238:1584—1586.
- Novotny, J., Brucolieri, R. E., and Saul, F. A. (1989) On the attribution of binding energy in antigen-antibody complexes McPC 603, D1.3, and HyHEL-5. *Biochemistry* 28:4735—4749.
- Novotny, J., Handschumacher, M., Haber, E., Brucolieri, R. E., Carlson, W. B., Fanning, D. W., Smith, J. A., and Rose, G. D. (1986) Antigenic determinants in proteins coincide with surface regions accessible to large probes (antibody domains). *Proc. Natl. Acad. Sci. U.S.A.* 83:226—230.
- Novotny, J., Handschumacher, M. a static surface property. *Immuno*
- Oertle, M., Immergluck, K., Pat four discontiguous antigenic de: 182:699—704.
- Padlan, E. A., Silverton, E. W., an antibody-antigen complex: complex. *Proc. Natl. Acad. Sc*
- Palfreyman, J. W., Aitcheson, production of polypeptide spec immunogens. *J. Immunol. Met*
- Parker, J. M. R., Guo, D., and Ho from high-performance liquid of predicted surface residues w *Biochemistry* 25:5425—5432.
- Parry, N. R., Syred, A., Rowlan of anti-peptide antibodies recce *munology* 64:567—572.
- Pellequer, J. L., Westhof, E., an methods for predicting the loc primary structures. *Meth. Enzy*
- Plaué, S. (1990) Synthesis of cycl of hemagglutinin of influenza
- Pollock, R. P., Teillaud, J. L., a powerful tool for selecting an *Ann. Rev. Microbiol.* 38:389-
- Quesniaux, V. F. J., Schmitter, D. (1990) Monoclonal antibodies tibody populations present in 27:227—236.
- Roehrig, J. T., Hunt, A. R., Joh peptides derived from the dedi Murray Valley encephalitis vir
- Rose, G. D., Geselowitz, A. R. (1985b) Hydrophobicity of a 229:834—838.
- Rose, G. D., Giersch, L. M., and *Adv. Protein Chem.* 37:1—105
- Sasaki, A., Mikawa, Y., Sakamoto Computer graphic analysis of an 25:157—163.
- Satterthwait, A. C., Arrhenius, T and Lerner, R. A. (1989) Th including a malaria peptide, fo B 323:565—572.
- Schaaper, M. M., Lankhof, H., Pe of antipeptide immune respons carrier protein. *Mol. Immunol.*

- cing heteroclitic bacteriophage an-
- , Neri, P., and Antoni, G. (1987) Conjugation enhances antibody 14:297—303.
- , R. J. (1987) The structural basis *ophys. Chem.* 16:139—159.
- lation of the topography and deter- oprotein of Saint Louis encephalitis lies. *J. Immunol.* 132:1533—1537.
- inivirus serotypes neutralized by 6—738.
- 986) Efficient mapping of protein *U.S.A.* 83:7013-7017.
- vier, V., Guetard, D., Bahraoui, anti-human immunodeficiency virus used with recombinant HIV-hepatitis *Sci. U.S.A.* 85:7957—7961.
- I. H. V. (1979) Immunochemical tion of five antigenic regions in the
- genmortel, M. H. V. (1986) Com- genic regions in histone H2A. *Mol.*
- I., Briand, J. P., and Van Regen- and protective capacity of a cyclic a virus haemagglutinin. *Vaccine*
- ls: a way for creating novel proteins.
- N., Martyn, J., Murray, J. M., ibodies elicited by influenza virus epresenting putative antigenic sites.
- , Reisfeld, R. A., Wilson, I. A., ation of protein-reactive antibodies implications for the structural basis *U.S.A.* 80:4949—4953.
- ughton, R. A., and Lerner, R. A. peptides representing the large gly- *Proc. Natl. Acad. Sci. U.S.A.*
- Handschenacher, M., and Haber, cience 238:1584—1586.
- 1989) On the attribution of binding 03, D1.3, and HyHEL-5. *Biochem-*
- Brucolieri, R. E., Carlson, W. B., (1986) Antigenic determinants in to large probes (antibody domains).
- Novotny, J., Handschenacher, M., and Brucolieri, R. E. (1987a) Protein antigenicity: a static surface property. *Immunol. Today* 8:26—31.
- Oertel, M., Immergluck, K., Paterson, Y., and Bosshard, H. R. (1989) Mapping of four discontiguous antigenic determinants on horse cytochrome c. *Eur. J. Biochem.* 182:699—704.
- Padlan, E. A., Silverton, E. W., Sheriff, S., and Cohen, G. H. (1989) Structure of an antibody-antigen complex: crystal structure of the HyHEL-10 Fab-lysozyme complex. *Proc. Natl. Acad. Sci. U.S.A.* 86:5938—5942.
- Palfreyman, J. W., Aitcheson, T. C., and Taylor, P. (1984) Guidelines for the production of polypeptide specific antisera using small synthetic oligopeptides as immunogens. *J. Immunol. Meth.* 75:383—393.
- Parker, J. M. R., Guo, D., and Hodges, R. S. (1986) New hydrophilicity scale derived from high-performance liquid chromatography peptide retention data: correlation of predicted surface residues with antigenicity and X-ray-derived accessible sites. *Biochemistry* 25:5425—5432.
- Parry, N. R., Syred, A., Rowlands, D. J., and Brown, F. (1988) A high proportion of anti-peptide antibodies recognize foot-and-mouth disease virus particles. *Im- munology* 64:567—572.
- Pellequer, J. L., Westhof, E., and Van Regenmortel, M. H. V. (1991) Overview of methods for predicting the location of continuous epitopes in proteins from their primary structures. *Meth. Enzymol.*, 203:176—201.
- Plaué, S. (1990) Synthesis of cyclic peptides on solid support. Application to analogs of hemagglutinin of influenza virus. *Int. J. Pept. Protein Res.* 35:510—517.
- Pollock, R. P., Teillaud, J. L., and Scharff, M. D. (1984) Monoclonal antibodies: a powerful tool for selecting and analyzing mutations in antigens and antibodies. *Annu. Rev. Microbiol.* 38:389—417.
- Quesniaux, V. F. J., Schmitter, D., Schreier, M. H., and Van Regenmortel, M. H. V. (1990) Monoclonal antibodies to cyclosporine are representative of the major antibody populations present in antisera of immunized mice. *Mol. Immunol.* 27:227—236.
- Roehrig, J. T., Hunt, A. R., Johnson, A. J., and Hawkes, R. A. (1989) Synthetic peptides derived from the deduced amino acid sequence of the E-glycoprotein of Murray Valley encephalitis virus elicit antiviral antibody. *Virology* 171:49—60.
- Rose, G. D., Geselowitz, A. R., Lesser, G. J., Lee, R. H., and Zehfus, M. H. (1985b) Hydrophobicity of amino acid residues in globular proteins. *Science* 229:834—838.
- Rose, G. D., Giersch, L. M., and Smith, J. A. (1985a) Turns in peptides and proteins. *Adv. Protein Chem.* 37:1—109.
- Sasaki, A., Mikawa, Y., Sakamoto, Y., Yamada, H., Ikeda, Y., and Ohno, T. (1988) Computer graphic analysis of antigenic sites on the insulin molecule. *Mol. Immunol.* 25:157—163.
- Satterthwait, A. C., Arrhenius, T., Hagopian, R. A., Zavala, F., Nussenzweig, V., and Lerner, R. A. (1989) The conformational restriction of synthetic peptides, including a malaria peptide, for use as immunogens. *Phil. Trans. R. Soc. London B* 323:565—572.
- Schaaper, M. M., Lankhof, H., Pujik, W. C., and Meloen, R. H. (1989) Manipulation of antipeptide immune response by varying the coupling of the peptide with the carrier protein. *Mol. Immunol.* 26:81—85.

- Schoofs, P. G., Geysen, H. M., Jackson, D., Brown, L. E., Tang, X. L., and White, D. O. (1988) Epitopes of an influenza viral peptide recognized by antibody at single amino acid resolution. *J. Immunol.* 140:611—616.
- Schulze-Gahmen, U., Klenk, H. D., and Beyreuther, K. (1986) Immunogenicity of loop-structured short synthetic peptides mimicking the antigenic site A of influenza virus hemagglutinin. *Eur. J. Biochem.* 159:283—289.
- Schulze-Gahmen, U., Rini, J. M., Arevalo, J., Stura, E. A., Kenten, J. H., and Wilson, I. A. (1988) Preliminary crystallographic data, primary sequence, and binding data for an anti-peptide Fab and its complex with a synthetic peptide from influenza virus hemagglutinin. *J. Biol. Chem.* 263:17100—17105.
- Scibienki, R. J. (1973) Denaturation of lysozyme by Freund's complete adjuvant. *J. Immunol.* 111:114—120.
- Sheriff, S., Silverton, E. W., Padlan, E. A., Cohen, G. H., Smith-Gill, S., Finzel, B. C., and Davies, D. R. (1987) Three-dimensional structure of an antibody-antigen complex. *Proc. Natl. Acad. Sci. U.S.A.* 84:8075—8079.
- Shi, P. T., Riehm, J. P., Todd, P. E. E., and Leach, S. J. (1984) The antigenicity of myoglobin-related peptides synthesised on polyacrylamide and polystyrene resin supports. *Mol. Immunol.* 21:489—496.
- Smith, A. M., and Benjamin, D. C. (1991) The antigenic surface of staphylococcal nuclease. II. Analysis of the N-1 epitope by site-directed mutagenesis. *J. Immunol.* 146:1259—1264.
- Smith, A. M., Woodward, M. P., Hershey, C. W., Hershey, E. D., and Benjamin, D. C. (1991) The antigenic surface of staphylococcal nuclease. I. Mapping epitopes by site-directed mutagenesis. *J. Immunol.* 146: 1254—1258.
- Smyth, M. S., Hoey, E. M., Trudgett, A., Martin, S. J., and Brown, F. (1990) Chemically synthesized peptides elicit neutralizing antibody to bovine enterovirus. *J. Gen. Virol.* 71:231—234.
- Soderquist, M. E., and Walton, A. G. (1980) Structural changes in proteins adsorbed on polymer surfaces. *J. Colloid Interface Sci.* 75:386—397.
- Stanfield, R. L., Fieser, T. M., Lerner, R. A., and Wilson, I. A. (1990) Crystal structures of an antibody to a peptide and its complex with peptide antigen at 2.8 Å. *Science* 248:712—719.
- Stanley, K. K. (1983) Solubilization and immune-detection of β-galactosidase hybrid proteins carrying foreign antigenic determinants. *Nucl. Acids Res.* 11:4077—4092.
- Stura, E. A., Stanfield, R. L., Fieser, T. M., Balderas, R. S., Smith, L. R., Lerner, R. A., and Wilson, I. A. (1989) Preliminary crystallographic data and primary sequence for anti-peptide Fab B13I2 and its complex with the C-helix peptide from myohemerythrin. *J. Biol. Chem.* 264:15721—15725.
- Tainer, J. A., Getzoff, E. D., Paterson, Y., Olson, A. J., and Lerner, R. A. (1985) The atomic mobility component of protein antigenicity. *Annu. Rev. Immunol.* 3:501—535.
- Tam, J. P. (1988) Synthetic peptide vaccine design: synthesis and properties of a high-density multiple antigenic peptide system. *Proc. Natl. Acad. Sci. U.S.A.* 85:5409—5413.
- Thornton, J. M., Edwards, M. S., Taylor, W. R., and Barlow, D. J. (1986) Location of "continuous" antigenic determinants in the protruding regions of proteins. *EMBO J.* 5:409—413.
- Thornton, J. M., and Sibanda, B. L. (1983) Amino and carboxy-terminal regions in globular proteins. *J. Mol. Biol.* 167:443—460.
- Trifilieff, E., Dubs, M. C., and V reactivity potential of synthetic *Immunol.* 28:889—896.
- Tulip, W. R., Varghese, J. N., Colman, P. M. (1989) Crystal *Cold Spring Harbor Symp. Qua*
- Underwood, P. A. (1982) Mapping Hong Kong influenza(H3N2) st *J. Gen. Virol.* 62:153—169.
- Underwood, P. A. (1985) Theoretical antibodies to detect antigenic particular reference to heterospi
- Van Regenmortel, M. H. V. (1988) antibodies. In *Hybridoma Techni* J. Stern, H. R. Gamble, eds.),
- Van Regenmortel, M. H. V. (198 tigenicity? *Trends Biochem.* Sc
- Van Regenmortel, M. H. V. (198 peptides: new insights and appl
- Van Regenmortel, M. H. V. (1989 epitopes. *Phil. Trans. R. Soc.*
- Van Regenmortel, M. H. V. (1989 of protein antigenicity. *Immuno*
- Van Regenmortel, M. H. V., Briai polypeptides as antigens. In *Lar Biology*, Vol. 19, Elsevier, An
- Van Regenmortel, M. H. V., and prediction methods for locating 17:95—108.
- Walter, G. (1986) Production an *Immunol. Meth.* 88:149—161.
- Welling, G. W., Weijer, W. J., Prediction of sequential antigen
- Westhof, E., Altschuh, D., Moras and Van Regenmortel, M. H. V. and the location of antigenic deter
- Wiley, D. C., and Skehel, J. J. (19 membrane glycoprotein of influ
- Wilson, I. A., Haft, D. H., Tainer S. (1985) Identical short peptide conformations: a testing ground *Acad. Sci. U.S.A.* 82:5255—52
- Wilson, I. A., Niman, H. L., M. L., and Lerner, R. A. (19 protein. *Cell* 37:767—778.
- Young, R. A., and Davis, R. W. (probes. *Proc. Natl. Acad. Sci.*

- Brown, L. E., Tang, X. L., and White, peptide recognized by antibody at single —616.
- Reutter, K. (1986) Immunogenicity of blocking the antigenic site A of influenza 283—289.
- J., Stura, E. A., Kenten, J. H., and graphic data, primary sequence, and complex with a synthetic peptide from n. 263:17100—17105.
- zyme by Freund's complete adjuvant.
- Cohen, G. H., Smith-Gill, S., Finzel, isional structure of an antibody-antigen 8075—8079.
- Leach, S. J. (1984) The antigenicity polyacrylamide and polystyrene resin
- the antigenic surface of staphylococcal site-directed mutagenesis. *J. Immunol.*
- W., Hershey, E. D., and Benjamin, ococcal nuclease. I. Mapping epitopes 16: 1254—1258.
- Martin, S. J., and Brown, F. (1990) lizing antibody to bovine enterovirus.
- structural changes in proteins adsorbed 75:386—397.
- ., and Wilson, I. A. (1990) Crystal complex with peptide antigen at 2.8
- re-detection of β -galactosidase hybrid ats. *Nucl. Acids Res.* 11:4077—4092.
- Salderas, R. S., Smith, L. R., Lerner, try crystallographic data and primary complex with the C-helix peptide from —15725.
- son, A. J., and Lerner, R. A. (1985) antigenicity. *Annu. Rev. Immunol.*
- gn: synthesis and properties of a high- n. *Proc. Natl. Acad. Sci. U.S.A.*
- ., and Barlow, D. J. (1986) Location protruding regions of proteins. *EMBO*
- nino and carboxy-terminal regions in 50.
- Trifilieff, E., Dubs, M. C., and Van Regenmortel, M. H. V. (1991) Antigenic cross-reactivity potential of synthetic peptides immobilized on polyethylene rods. *Mol. Immunol.* 28:889—896.
- Tulip, W. R., Varghese, J. N., Webster, R. G., Air, G. M., Laver, W. G., and Colman, P. M. (1989) Crystal structures of neuraminidase-antibody complexes. *Cold Spring Harbor Symp. Quant. Biol.* LIV:257—263.
- Underwood, P. A. (1982) Mapping of antigenic changes in the haemagglutinin of Hong Kong influenza(H3N2) strains using a large panel of monoclonal antibodies. *J. Gen. Virol.* 62:153—169.
- Underwood, P. A. (1985) Theoretical considerations of the ability of monoclonal antibodies to detect antigenic differences between closely related variants, with particular reference to heterospecific reactions. *J. Immunol. Meth.* 85:295—307.
- Van Regenmortel, M. H. V. (1984) Molecular dissection of antigens by monoclonal antibodies. In *Hybridoma Technology in Agricultural and Veterinary Research* (N. J. Stern, H. R. Gamble, eds.), Rowman and Allanheld, Totowa, NJ, pp. 43—82.
- Van Regenmortel, M. H. V. (1986) Which structural features determine protein antigenicity? *Trends Biochem. Sci.* 11:36—39.
- Van Regenmortel, M. H. V. (1987) Antigenic cross-reactivity between proteins and peptides: new insights and applications. *Trends Biochem. Sci.* 12:237—240.
- Van Regenmortel, M. H. V. (1989a) The concept and operational definition of protein epitopes. *Phil. Trans. R. Soc. London B* 323:451.
- Van Regenmortel, M. H. V. (1989b) Structural and functional approaches to the study of protein antigenicity. *Immunol. Today* 10:266—272.
- Van Regenmortel, M. H. V., Briand, J. P., Muller, S., and Plaué, S. (1988) Synthetic polypeptides as antigens. In *Laboratory Techniques in Biochemistry and Molecular Biology*, Vol. 19, Elsevier, Amsterdam, pp. 1—227.
- Van Regenmortel, M. H. V., and Daney de Marcillac, G. (1988) An assessment of prediction methods for locating continuous epitopes in proteins. *Immunol. Lett.* 17:95—108.
- Walter, G. (1986) Production and use of antibodies against synthetic peptides. *J. Immunol. Meth.* 88:149—161.
- Welling, G. W., Weijer, W. J., Van der Zee, R., and Welling-Wester, S. (1985) Prediction of sequential antigenic regions in proteins. *FEBS Lett.* 188:215—218.
- Westhof, E., Altschuh, D., Moras, D., Bloomer, A. C., Mondragon, A., Klug, A., and Van Regenmortel, M. H. V. (1984) Correlation between segmental mobility and the location of antigenic determinants in proteins. *Nature (London)* 311:123—126.
- Wiley, D. C., and Skehel, J. J. (1987) The structure and function of the hemagglutinin membrane glycoprotein of influenza virus. *Annu. Rev. Biochem.* 56:365—394.
- Wilson, I. A., Haft, D. H., Tainer, J. A., Getzoff, E. D., Lerner, R. A., and Brenner, S. (1985) Identical short peptide sequences in unrelated proteins can have different conformations: a testing ground for theories of immune recognition. *Proc. Natl. Acad. Sci. U.S.A.* 82:5255—5259.
- Wilson, I. A., Niman, H. L., Houghten, R. A., Cherenson, A. R., Connolly, M. L., and Lerner, R. A. (1984) The structure of an antigenic determinant in a protein. *Cell* 37:767—778.
- Young, R. A., and Davis, R. W. (1983) Efficient isolation of genes by using antibody probes. *Proc. Natl. Acad. Sci. U.S.A.* 80:1194—1198.